Population genetics, phylogeography, and morphology of *Notropis stramineus*

By

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Abstract

Notropis stramineus, also known as the Sand Shiner, is a widespread, common minnow species in eastern North America that inhabits low-gradient streams and lake margins with sand or cobble substrate. The morphology of *N. stramineus* exhibits a distinct pattern of geographical variation which has compelled authors to recognize two subspecies within this species. In this study, I investigated both the genetic and morphological diversity of *N. stramineus*. First, I used microsatellite markers to investigate intraspecific genetic variation within a single stream corridor to evaluate population substructure and barriers to dispersal for *N. stramineus.* Population genetic analyses indicated that there is no genetic structuring between populations of *N. stramineus* in the Kansas River and that fish in the study area form one panmictic population. Additionally, the two dams within the study area do not appear to restrict dispersal among sample sites. Geographic distance, at least at the scale of this study, also does not appear to be a barrier to dispersal as there was no genetic signal of isolation by distance. Next, I investigated genetic variation across the majority of the distribution of *N. stramineus* using phylogeographic techniques to test the validity that nominal subspecies are reflective of evolutionary lineages. I also used this data to address hypotheses for the origin of a Texas disjunct population. Phylogeographic analyses rejected the validity of subspecies and instead revealed the existence of five exclusive evolutionary groups, possibly cryptic species, within the nominal *N. stramineus*. The analyses also support a hypothesis that the disjunct Texas population originated from the Ancestral Plains Stream sometime during the Pleistocene. Finally, I tested the hypothesis that the morphology of the exclusive evolutionary groups in the Great Plains has diverged and is concordant with genetic divergence. Discriminant Function Analysis of 29 morphological shape variables showed that each exclusive evolutionary group in the Great Plains has a unique morphological shape. These findings show that morphological divergence in *N. stramineus* is concordant with genetic divergence and highly supports the recognition of the exclusive evolutionary groups revealed by phylogeographic analyses as distinct species.

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Introduction

Population connectivity in stream-dwelling organisms acts to maintain gene flow and decrease population structuring within species. When connectivity between populations is inhibited, divergence can occur which then results in population substructure (Dehais et al. 2010). If an aquatic species inhabits a patchy environment or has poor dispersal ability, gene flow is reduced and populations can genetically diverge even though they are within the same drainage. The importance of movement along a stream corridor for population dynamics of stream fishes is an increasing area of study and is reviewed in Koizumi (2011). Two common obstructions to connectivity for freshwater fishes are physical barriers and waterway distance. Physical barriers, such as natural falls or human-made dams, can fragment fish habitat and impede natural gene flow which results in structured populations (Koizumi 2011). Because waterways are becoming more impounded, habitat fragmentation is a major concern for stream-dwelling fishes.

Connectivity between populations of stream fishes can also be decreased if the dispersal ability of a fish species is only moderate; thus, the species experiences decreased gene flow with increased geographic distance. This type of scenario would result in a genetic signature of isolation by distance (sensu Wright 1943). In this study, I address the effects of both physical barriers to population connectivity (e.g. dams) and waterway distance on gene flow in the widespread fish species, *Notropis stramineus*.

I examined the population genetic structure of *N. stramineus* in the Kansas and Smoky Hill Rivers using 7 microsatellite loci and tested the hypothesis that samples of *N. stramineus* show genetic differentiation in this drainage basin. If *N. stramineus* has high dispersal ability and moves freely within the sampled area of the Kansas River, the

hypothesis will not be supported. If the dispersal ability of *N. stramineus* were high, but not high enough to overcome the isolating effect of dams on the river, then I would expect to see genetic differentiation only between the isolated samples and the contiguous distribution. Alternatively, if *N. stramineus* only has a medium or low dispersal ability, then the analysis could reveal isolation by distance or significant divergence between all samples.

Study Species

Notropis stramineus (Cope), also known as the Sand Shiner, is a common minnow found in clear, low gradient streams of all sizes that have cobble or sand substrate (Tanyolac 1973). It has a widespread distribution in North America that ranges from the Rocky Mountains eastward to the western edges of Pennsylvania and New York State and from the Great Lakes south to Tennessee and also includes two disjunct populations, one in southern Texas ranging from the Trinity River to the Rio Grande on the Gulf slope, and the other in the drainages of the Tennessee and Cumberland Rivers (Page and Burr 1991). Nothing is known about the dispersal ability of *N. stramineus*.

Study Area

The Kansas River, a 7th order tributary of the Missouri River, is a sand-bed river that is typically shallow (< 1.5 m) and has a braided channel (Makinster and Paukert 2008). Ephemeral sandbars are common occurrences in the river while permanent islands with long-lasting vegetation are not (Moore and Thorp 2008). Mean annual stream flow in 2004 was 124.8 m^3s^{-1} but was highly variable (Thorp and Mantovani 2005).

The Kansas River begins at the junction of the Smokey Hill River and the Republican River near Junction City, KS and extends 274 km to its confluence with the Missouri River in Kansas City, KS (Paukert et al. 2008). Tributaries extend westward into Colorado and northward into Nebraska such that the Kansas River drains ~ 159,000 km^2 of the Great Plains (Galat et al. 2005). While dams and reservoirs are common occurrences in tributaries of the Kansas River, no flood control structures exist in the mainstem except for occasional bank stabilization (Moore and Thorp 2008). The main channel has two weirs that divert water for city water supplies (Topeka, KS and Kansas City, KS) and one low-head hydroelectric dam (Bowersock Dam; Lawrence, KS). The dam creates a low velocity, reservoir-like habitat for 5-6 km upstream (Eitzmann et al. 2007). While the weirs do not appear to restrict fish movement in the Kansas River, Quist and Guy (1999) demonstrated that the Bowersock Dam might partially restrict upstream movements of some fishes when discharge is low.

The Smoky Hill River can be considered a westward extension of the Kansas River, and its inclusion in this study allows for the consideration of a longer contiguous stream segment. Two major impoundments occur on the Smoky Hill River, the Cedar Bluff Reservoir near Trego Center and Kanopolis Lake near Kanopolis.

Materials and Methods

Population sampling and DNA isolation

I examined 105 specimens of *N. stramineus* from 6 sites (collected by myself and others during 1997-2009, preserved in 95% ethanol) in the Kansas River and its tributaries (Figure 1). Three of the sites occur the mainstem Kansas River, two sites occur in the mainstem Smoky Hill River, and one site is from a southern tributary of the Kansas River (Mill Creek). The westernmost sample site is isolated from the other sample sites by Kanopolis Lake while the easternmost sample is isolated from the rest of the samples by Bowersock Dam. The four intervening sample sites are unhindered by obvious obstacles to migration. I removed muscle tissue from the side of each specimen and extracted DNA using a DNeasy Tissue Kit (Qiagen). Extracted DNA was stored at -20 ° C until needed. A list of specimens, collections dates, and complete locality information is presented in Appendix 1 (Material Examined).

Laboratory Procedures

I screened 16 microsatellites, originally developed for *Notropis mekistocholas* (Burridge and Gold 2003, Gold et al. 2004), for use in *N. stramineus* using a 3-primer system in which one locus-specific primer is untagged, one locus specific primer is tagged with a CAG sequence (5-CAGTCGGGCGTCATCA-3'), and the third primer is the CAG sequence with a 5' 6-FAM fluorescent label (Boutin-Ganache et al. 2001, Skalski and Grose 2006). Screening was performed on specimens from the Lawrence sample site. PCR reactions (15 µL) contained ~ 10ng DNA in 1X PCR buffer, 1.5 mM $MgCl^2$, 0.32 mM dNTP mix, 0.25 µM of unlabeled primer, 0.025uM CAG tagged primer, 0.25 µM 6-FAM labeled CAG

primer and 0.5 U of iTaq DNA Polymerase (Bio-Rad Corporation). Touchdown PCR was employed with an initial denaturation at 94 °C for 2 min, 21 cycles of denaturation at 94 °C for 20 sec, annealing at T_a for 20 sec, and extension at 72 °C for 30 sec (where T_a was decreased by 0.5 °C each cycle, followed by 10 cycles of 94 °C for 20 sec, annealing at the final T_a for 20 sec, and extension at 72 °C for 30 sec, and ending with a final extension at 72 °C for 10 min. Primer sequences and initial T_a used for screening are listed in Table 1.

PCR conditions were optimized for the seven polymorphic microsatellite loci and the loci amplified in all specimens using a thermocycling protocol with an initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at T_a for 30 sec, and extension at 72 °C for 60 sec and ending with a final extension at 72 °C for 10 min. I again used the 3-primer system described above but I used 4 different fluorescent dyes (6-FAM, VIC, NED, or PET) to enable the use of post-amplification multiplexing. PCR reactions (15 µL) contained ~ 10ng DNA in 1X PCR buffer, 1.5 mM $MgCl^{2+}$, 0.32 mM dNTP mix, 0.25 µM of unlabeled primer, 0.025uM CAG tagged primer, 0.25 µM fluorescently-labeled CAG primer and 0.5 U of Biolase™ DNA Polymerase (Bioline USA Inc.). Optimized annealing temperatures used for each microsatellite marker are listed in Table 1.

The samples were multiplexed for fragment analysis by combining 5 µL of PCR product from 4 differently labeled microsatellites to form a pooled sample. The pooled samples were purified using Sephadex™ (GE Healthcare Life Sciences) and then sized by capillary electrophoresis at the University of Kansas DNA Sequencing Lab (Lawrence, KS). Fragments were manually genotyped fragments using Peak Scanner™ v1.0 (Applied Biosystems).

Population Genetic Analysis

Genetic diversity was measured as number of alleles per locus, observed heterozygosity, and the expected heterozygosity; the inbreeding coefficient F_{IS} was also calculated using the software Arelquin 3.5.1.3 (Excoffier and Lischer 2010). F_{IS} was estimated under both the infinite alleles model (IAM), which produces F-statistics, and the stepwise mutation model (SMM), which produces R-statistics. While stepwise mutation appears to be the dominant force in microsatellite evolution (Eisen 1999, Ellegren 2004), non-stepwise mutation processes, such as point mutations, do occur in these loci (Richard and Paques 2000). Balloux et al. (2000) demonstrated that even a slight deviation from the SMM can cause estimates of R_{st} to be unreliable. Additionally, numerous empirical studies of population structure have concluded that many microsatellite loci do not fit the SMM and that the IAM performs better for these loci (e.g. Estoup et al. 1995a, Estoup et al. 1995b, Goodman 1998, Neff et al. 1999, Ross et al. 1999). Because neither the IAM nor SMM satisfactorily explain the mutation process of microsatellites, and because I had no *a priori* reason to overwhelmingly prefer either model for the markers used in this study, I used both F_{st} and R_{st} values.

I tested for linkage disequilibrium between all pairs of loci within each sample site with Slatkin's (1994) extension of Fisher's exact probability test on contingency tables using 10,000 steps in the Markov chain and 1000 dememorization steps as implemented in Arlequin. The statistical significance level was adjusted for simultaneous tests to maintain a within-sample Type-I error rate of 0.05% by using a sequential Bonferroni correction (Rice 1989) resulting in a significance level per test of P=0.0004.

I tested sample sites for deviation from Hardy-Weinberg equilibrium at each locus using a modified version of the Markov-chain random walk algorithm of Guo and Thompson (1992) using 100,000 steps in the Markov chain and 1000 dememorization steps as implemented in Arlequin. Again, the statistical significance level was adjusted for simultaneous tests to maintain a within-sample Type-I error rate of 0.05% by using a sequential Bonferroni correction (Rice 1989) resulting in a significance level per test of P=0.0012.

Population genetic structure was measured using an analysis of molecular variance (AMOVA; Excoffier et al. 1992) as executed in Arlequin where diversity was partitioned hierarchically among sample sites, within sample sites, and within individuals. Because there is some missing data (i.e. amplification failure due to poor quality DNA), the most appropriate approach is a locus-by-locus AMOVA. In this approach, the fixation statistics are calculated using Wier and Cockerham's (1984) estimator of Wright's F-statistics (Wright 1951, 1965). A global AMOVA can then be computed that is a weighted average over all loci. The average is not an arithmetic mean; it is calculated by combining variance components at each level of the hierarchy. Variance components at a given level are summed in the numerator and denominator to produce fixation statistics as variance component ratios (Wier and Cockerham 1984, Wier 1996). The significance of the fixation indices was measured using the non-parametric permutation procedure of Excoffier et al. (1992) with 1000 permutations. To measure genetic divergence between sample sites, I computed population pairwise fixation indices. The significance of the population pairwise fixation indices was again measured using the non-parametric permutation procedure of Excoffier et al. (1992) with 1000 permutations. Finally, I used Arelquin to test

the hypothesis of isolation by distance using a Mantel test with 10000 random permutations between the matrix of stream distance and the matrix of pairwise genetic differentiation between sample sites. All calculations based on comparisons of fixation indices were done under both the IAM (F-statistics) and a SMM (R-statistics) as per the justifications noted above.

Results

The number of alleles per locus per sample site ranged from 2 to 21, and the average number of alleles per locus in each sample site ranged from 6.28 to 9.14 (Table 2). Private alleles (14 total) were present in 6 out of the 7 loci but were low in number and only found in one or two sample sites per locus. The most common alleles at each locus were shared in all samples. The average observed heterozygosity within each sample site was moderate and ranged from 0.51125 to 0.65714. The complete data set of microsatellite genotypes for each individual is given in Appendix 2.

Population specific F_{IS} values (average F_{IS}) ranged from -0.02304 to 0.23024 under the IAM and from -0.09953 to 0.37973 under the SMM (Table 2). Positive values of F_{IS} were indicative of a heterozygote deficiency while negative values of F_{IS} indicate heterozygote excess. No significant heterozygote excess was observed in any of the samples using either mutation model. Four samples showed significant levels of heterozygote deficiency ($F_{IS} > 0$; $P < 0.05$) before Bonferroni correction for multiple comparisons. Two sample sites (Manhattan and Enterprise) still exhibited significant heterozygote deficiency following Bonferroni correction ($F_{IS} > 1$; $\alpha=0.05$, $P < 0.0083$) but only under the IAM. Lack of statistically significant heterozygote deficiency between these two samples sites using the SMM can be explained by the high variance of R-statistics under the SMM (Estoup et al. 2002).

Twelve sample-locus combinations showed significant deviation from Hardy-Weinberg equilibrium ($\alpha=0.05$, $P < 0.05$, Table 2) but following Bonferroni correction, only two sample-loci combinations showed deviation from Hardy-Weinberg equilibrium ($\alpha=0.05$, $P < 0.0012$). Examination of these two loci in other samples does not seem to

indicate the presence of null alleles, as there were no amplifications failures for any individuals at these loci. Removal of these 2 loci did not markedly affect the outcomes of further analyses. Thus, both loci were both retained for further analysis.

Seven pairs of loci exhibited linkage disequilibrium in four samples (α=0.05, $P < 0.05$, Table 3) but none of the comparisons was significant following Bonferroni correction (α=0.05, $P < 0.0004$). Thus, all of the loci were found to be independent markers and were retained for further analysis.

Global analyses of molecular variance indicated that the vast majority of genetic variation was found within individuals with 82.6% under the IAM (Table 4) and 78.6% under the SMM (Table 5). Very little variation was found among sample sites with 0.03% under the IAM (Table 4) and 1.48% under the SMM (Table 5). F_{ST} values were extremely low and did not differ significantly from zero in either the locus-by locus AMOVA (Table 6) or the global AMOVA analyses under both the IAM (Table 4) and SMM (Table 5).

After Bonferroni correction, none of the population pairwise F_{st} values were significant and only one R_{st} value was significant (α=0.05, $P < 0.0033$; Table 7). This significant value was for the comparison between Topeka and Manhattan samples, two samples that are adjacent along the Kansas River. More distant sample sites did not show significant differentiation.

Mantel tests for isolation by distance were non-significant using both population pairwise F_{st} (P=0.92100) and R_{st} (P= 0.98500) values. This indicates that there is no population structuring correlated with geographical distance in *N. stramineus* samples in the Kansas River.

Discussion

Allelic diversity and average observed heterozygosities in this study are consistent with those reported for other freshwater fishes (DeWoody and Avise 2000). Of particular interest in this study are the significant positive values of F_{IS} found in four samples and by the global AMOVAs. Positive values of F_{IS} indicate heterozygote deficiency. Heterozygote deficiencies are not uncommon and may result from population substructure (Wahlund 1928, Chakraborty and Jin 1992). Because F_{ST} values in this study are effectively zero, this explanation (which depends of population differentiation) is not adequate. Another explanation for heterozygote deficiency is needed.

Heterozygote deficiency in the presence of panmixia can be caused by the sampling of shoaling kin (Krause et al. 2000, Castric et al. 2002). Individuals of *N. stramineus*, as in many other cyprinid species, shoal in groups. Several studies have found that some fish species, including several cyprinids, shoal with conspecifics (reviewed in Krause et al. 2000). Typical field sampling using seines with consecutive hauls in the same general area can capture individuals belonging to the same shoal. This is especially likely to happen if one sampling event collects enough individuals to preclude additional sampling. If shoals are comprised of closely related individuals, inbreeding will appear in the genetic signature (e.g. $F_{IS} > 0$) due to this non-random sampling. It is plausible that each site sampled in this study inadvertently contained closely related individuals of *N. stramineus*. Castric et al. (2002) had some success using the genetic relatedness of pairs of brook char to test the hypothesis that their samples contained closely related individuals. Future analysis of shoaling fish species should consider adapting this methodology.

Despite significant heterozygote deficiency, the results of this study indicate that samples of *N. stramineus* in the Kansas River and its tributaries are not genetically structured and show evidence of panmixia under both the IAM and the SMM. Global AMOVAs indicates that nearly all (>98%) of the genetic variation seen in the surveyed microsatellites is contained within individuals and samples. The only significant population pairwise comparison was found between the Topeka and Manhattan samples and only under the SMM. If one includes significant values before Bonferroni correction, three pairs of sample sites differ significantly (under both the IAM and SMM), and all involve the Topeka sample. These findings might be explained by the 3 private alleles found in locus 2 in the Topeka sample. These results could simply be artifacts of the sampling. There is no intuitive reason why the Topeka sample site (which is towards the center of the distribution) would be differentiated from other samples taken from the Kansas River. There are no barriers (physical or ecological) to dispersal either in or out of this sample site. If the Bowersock Dam creates a barrier to dispersal between the Topeka and Lawrence sample sites, there should have been also been a difference between Lawrence samples and all other upstream samples; this is not the case. Additionally, the Topeka sample is not genetically different from the furthest sample (Russell). While statistically significant, the R_{ST} values are low (< 0.1) and may have no biological meaning. Moreover, the slight distinctness of the Topeka sample is not enough to cause a significant R_{ST} to be recovered from the global AMOVA analysis. Therefore, I am confident that there is no significant genetic structuring among the studied samples.

The lack of population substructure and isolation by distance in *N. stramineus* in the Kansas River indicates that this species can move freely within the Kansas River, even in

the presence of fragmentation caused by dams and reservoirs. This finding is not surprising considering the large distribution and natural history of this species. *N. stramineus* is closely associated with sandbars (Tanyolac 1973) and has adapted to an ever-shifting habitat as sandbars move along river bottoms as river discharge varies. As the sandbars move, so do the fishes.

Several other studies have also failed to find significant genetic structure in populations of stream-dwelling fishes using microsatellite markers. Burridge and Gold (2003) investigated the population genetic structure of the endangered Cape Fear Shiner, *N. mekistocholas* in the Cape Fear drainage of North Carolina. Geographical distances between samples of *N. mekistocholas* were similar to distance between *N. stramineus* samples in this study. Despite the presence of many barriers (e.g. dams and natural falls) among populations, little genetic structure was found between *N. mekistocholas* sample sites. Young (2001) found that populations of the wide-ranging Emerald Shiner, *Notropis atherinoides*, are effectively panmictic in the Missouri River ranging from Montana to Missouri despite the presence of many large dams. The geographical distances between samples of *N. atherinoides* were far great than the distances between samples of *N. stramineus* in the present study. Wozney (2011) sampled genetic diversity in the highly migratory lake sturgeon, *Acipenser fulvescens*, in the entirety of the Ottawa River and found that the samples were panmictic despite numerous, large hydroelectric dams that function as barriers to migration. The geographical distances between samples of *A. fulvescens* were very similar to those between *N. stramineus* sample sites in this study while the dams were much larger.

Considering the present data, high historical and contemporary gene flow exists in *N. stramineus* in the Kansas River enabled by high dispersal. Physical barriers caused by dams and reservoirs do not appear to hinder gene flow. Additionally, waterway distance, at least at the scale in this study, does not impede the exchange of genes between distant sample sites. Samples of *N. stramineus* in the Kansas River are highly connected and behave as one panmictic population.

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Table 1
List of microsatellite loci, their corresponding primer sequences, and annealing temperatures used in this study.

Locus	Locus Name	T_a (°C) - screening	T_a (°C) - final amplification	PCR primer sequences (5'-3')
1	Nme 5B10.211	65	58	gacccgtacagtgctct
				gaaagtccccataaaaca*
2	Nme 2B10.232	65	42	aatgcaaaagcaactgtaatcaacta
				atggtcatggcctaaagcaatac*
3	Nme 6A7.93	65	56	caccaaactgtcattcaaataag
				gaccctgggcgttctctg*
4	Nme 30F12.208	60	56	tacatcatggccctaacaca*
				gggctaaaatttggacgaa
5	Nme 25C8.208	60	59	aaaaaggcctcccagtgc*
				aattatatgtcggtgaccagattg
6	Nme 24B6.211	65	57	ttgcaggggaaacatacc
				gaatgggccgttactctc*
7	Nme 2B10.166	60	54	cagaagtgccagcgagaatgt*
				tcaaagatgcccgcccctaata

* CAG-tagged primer

(T_a) - annealing temperature

Table 2
Individual microsatellite locus statistics for *N. stramineus* populations.

Sample	Par.	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7	Avg.
Lawrence	A	7[2]	16[1]	12[1]	11[1]	9[0]	4[1]	2[0]	8.714
N=19	H_O	0.4118	0.9474	0.7222	0.6316	0.5790	0.3684	0.0526	0.5304
	H_E	0.6524	0.9331	0.8841	0.8634	0.8279	0.6074	0.0526	0.6887
	P_{HW}	**0.0345**	0.8059	0.0755	**0.0020**	**0.0431**	**0.0036**	1.0000	---
	R_{IS}	0.7876	0.0783	0.4659	0.3186	0.2443	0.5804	0.0000	0.1891
	F_{IS}	0.0376	-0.0157	0.1875	0.2740	0.3065	0.4000	0.0000	**0.2302***
Topeka	A	3[0]	21[3]	12[0]	12[0]	10[2]	3[0]	3[0]	9.143
N=20	H_O	0.6000	0.9000	0.6000	0.8500	0.7000	0.3000	0.2000	0.5929
	H_E	0.5372	0.9615	0.8308	0.8923	0.8551	0.2680	0.2718	0.6595
	P_{HW}	0.6519	0.3757	**0.0045**	0.7245	0.1620	1.0000	0.1814	---
	R_{IS}	-0.2089	0.1492	0.0127	0.1758	0.0948	-0.1325	0.1318	0.1444
	F_{IS}	-0.1204	0.0656	0.2830	0.0486	0.1853	-0.1232	0.2692	0.0638
Mill Creek	A	3[0]	16[0]	9[0]	13[0]	8[2]	3[0]	3[0]	7.857
N=17	H_O	0.4706	0.8824	0.5714	0.8235	0.6471	0.5294	0.1765	0.5858
	H_E	0.4902	0.9483	0.8413	0.9055	0.7968	0.5187	0.2692	0.6814
	P_{HW}	1.0000	0.4933	0.0258	0.2567	0.0945	1.0000	**0.0421**	---
	R_{IS}	0.1579	0.1695	0.5198	0.5069	-0.1555	-0.0127	0.0400	0.2292
	F_{IS}	0.0412	0.0716	0.3290	0.0931	0.1927	-0.0213	0.3514	**0.1045**
Manhattan	A	2[0]	16[0]	10[0]	11[0]	9[0]	4[0]	4[0]	8.000
N=20	H_O	0.4118	0.7000	0.6111	0.7059	0.6000	0.3500	0.2000	0.5113
	H_E	0.4866	0.9321	0.8444	0.9020	0.8449	0.5846	0.2346	0.6899
	P_{HW}	0.6245	**0.0121**	0.0727	**0.0155**	**0.0005***	0.0523	0.2455	---
	R_{IS}	0.1579	0.2634	0.5508	0.7001	0.5358	0.3091	0.3962	**0.3797**
	F_{IS}	0.1579	0.2539	0.2822	0.2227	0.2952	0.4075	0.1508	**0.2256***
Enterprise	A	5[0]	15[0]	11[1]	13[0]	7[0]	4[0]	4[0]	8.429
N=20	H_O	0.4737	0.8421	0.5263	0.8421	0.4737	0.4737	0.1579	0.5414
	H_E	0.5292	0.9289	0.8649	0.9218	0.7866	0.5178	0.1536	0.6718
	P_{HW}	0.3308	0.5742	**0.0011***	**0.0427**	**0.0019**	0.5511	1.0000	---
	R_{IS}	0.2157	-0.2805	-0.0501	-0.1065	0.6997	-0.1180	-0.0456	-0.0995
	F_{IS}	0.1074	0.0958	0.3980	0.0886	0.4044	0.0873	-0.0286	**0.1533***
Russell	A	3[0]	12[0]	7[0]	9[0]	7[0]	3[0]	3[0]	6.286
N=10	H_O	0.4000	1.0000	0.8000	0.9000	1.0000	0.3000	0.2000	0.6571
	H_E	0.4684	0.9477	0.8526	0.7947	0.8684	0.5316	0.1947	0.6655
	P_{HW}	1.0000	0.5264	0.6490	0.5189	0.9876	0.1081	1.0000	---
	R_{IS}	0.2822	-0.0791	0.5573	0.3214	-0.4153	0.0471	-0.0541	-0.0353
	F_{IS}	0.1529	-0.0588	0.0649	-0.1409	-0.1613	0.4490	-0.0286	-0.0230
Average R_{IS}		0.3159	0.1042	0.3259	0.3331	0.1210	0.1793	0.1343	---
Average F_{IS}		0.1197	0.0830	0.2722	0.1188	0.2343	0.2250	0.1779	---

N – number of individuals in the sample; A – observed number of alleles per locus with number of private alleles in brackets; H_o – observed heterozygosity; H_E – expected heterozygosity; P_{HW} – probability of departure from Hardy-Weinberg equilibrium; R_{IS} – inbreeding coefficient based on R_{ST} (assumes step-wise mutation model); F_{IS} – inbreeding coefficient based on F_{ST} (assumes infinite alleles model).

Table 3
Pairwise linkage disequilibrium exact p-values for each population. Bold values are statistically significant ($p \leq 0.05$) before Bonferonni correction. No values were statistically significant following Bonferonni correction for simultaneous tests.

	Population					
Loci Pair	Lawrence	Topeka	Mill Creek	Manhattan	Enterprise	Russell
1, 2	0.849802	0.500099	0.556040	0.912574	0.426535	0.345545
1, 3	0.203861	0.464356	0.560792	0.148515	0.615743	0.868020
1, 4	**0.046733**	0.342376	0.229802	0.065446	0.387723	0.243267
1, 5	0.763267	0.440792	0.768911	0.815743	0.822376	0.077525
1, 6	0.194554	1.000000	0.327822	0.318713	0.968020	0.489010
1, 7	0.497822	1.000000	0.254257	0.806832	0.734554	**0.034158**
2, 3	0.073960	0.833960	0.495644	0.555149	0.742475	0.337624
2, 4	0.164752	0.858614	0.889010	0.135644	0.776634	1.000000
2, 5	0.176040	0.068317	**0.034950**	0.779505	0.461683	1.000000
2, 6	**0.010990**	0.507030	0.667426	0.213366	**0.024059**	0.832871
2, 7	0.681188	0.665941	0.220792	0.208119	0.731881	0.433564
3, 4	**0.000693**	0.929208	0.330990	0.450495	0.239406	0.701980
3, 5	0.722772	0.569010	0.498317	0.323069	0.105050	0.992376
3, 6	0.233564	1.000000	0.081386	0.240396	0.214752	0.653564
3, 7	0.105050	0.839604	1.000000	0.278416	0.471980	0.788911
4, 5	0.160495	0.199010	0.076238	0.472574	0.374851	0.761089
4, 6	0.119406	0.489109	0.805446	0.060594	0.187228	0.962079
4, 7	0.370495	0.693663	0.228020	0.635545	0.716535	**0.020198**
5, 6	**0.025248**	0.477624	0.328911	0.313960	0.075743	0.674653
5, 7	1.000000	0.736436	0.812574	0.085941	0.570396	0.438713
6, 7	0.181584	0.449406	0.736832	0.267624	1.000000	0.590693

Table 4
Global AMOVA design and results using the infinite alleles model.

Source of variation	Sum of squares	Variance components	Percentage variation	F	P
Among populations	14.07700	0.00067	0.02804	0.00028	1.00000
Among individuals within populations	269.82600	0.41291	17.336	0.17341	0.00000
Within individuals	202.00000	1.96822	82.63595	0.17364	0.00000
Total	485.90300	2.38179			

Table 5
Global AMOVA design and results using the stepwise mutation model.

Source of variation	Sum of squares	Variance components	Percentage of variation	F	P
Among populations	2220.05200	3.8805	1.47988	0.01480	0.54839
Among individuals within populations	29971.94300	51.81842	19.76169	0.20059	0.00000
Within individuals	21272.00000	206.51757	78.75842	0.21242	0.00000
Total	53463.99500	262.21648			

Table 6
Locus by locus AMOVA using both F_{ST} and R_{ST}. The average for each value is a weighted average over all loci. None of the values were significant.

Locus	F_{st}	P-value	R_{st}	P-value
1	0.01083	0.59726	0.06258	0.06940
2	-0.00322	1.00000	0.03879	0.12121
3	-0.00487	1.00000	-0.01834	0.99609
4	0.00403	0.98436	-0.00539	0.88661
5	-0.00266	0.99902	-0.01667	1.00000
6	0.00666	0.70674	-0.0165	1.00000
7	-0.0107	1.00000	-0.00737	0.91300
Average	0.00028	1.00000	0.0148	0.54839

Table 7 Pairwise Population fixation indices.

	Lawrence	Topeka	Mill Creek	Manhattan	Enterprise	Russell
Lawrence	-	**0.01536**	0.00063	0.00264	-0.00535	0.00655
Topeka	**0.05167**	-	0.00343	**0.01059**	**0.01781**	0.01599
Mill Creek	-0.00892	**0.03913**	-	-0.00738	-0.00312	-0.00949
Manhattan	0.02645	**0.09425***	-0.02792	-	-0.00104	0.00423
Enterprise	0.00978	0.0176	-0.01496	0.03663	-	0.00082
Russell	-0.03142	-0.01078	-0.022	0.0057	-0.00908	-

F_{st} above the diagonal, R_{st} below the diagonal. Values in bold are statistically significant ($p \leq 0.05$) before Bonferonni correction. The * indicates statistical significance after Bonferonni correction for 15 simultaneous tests.

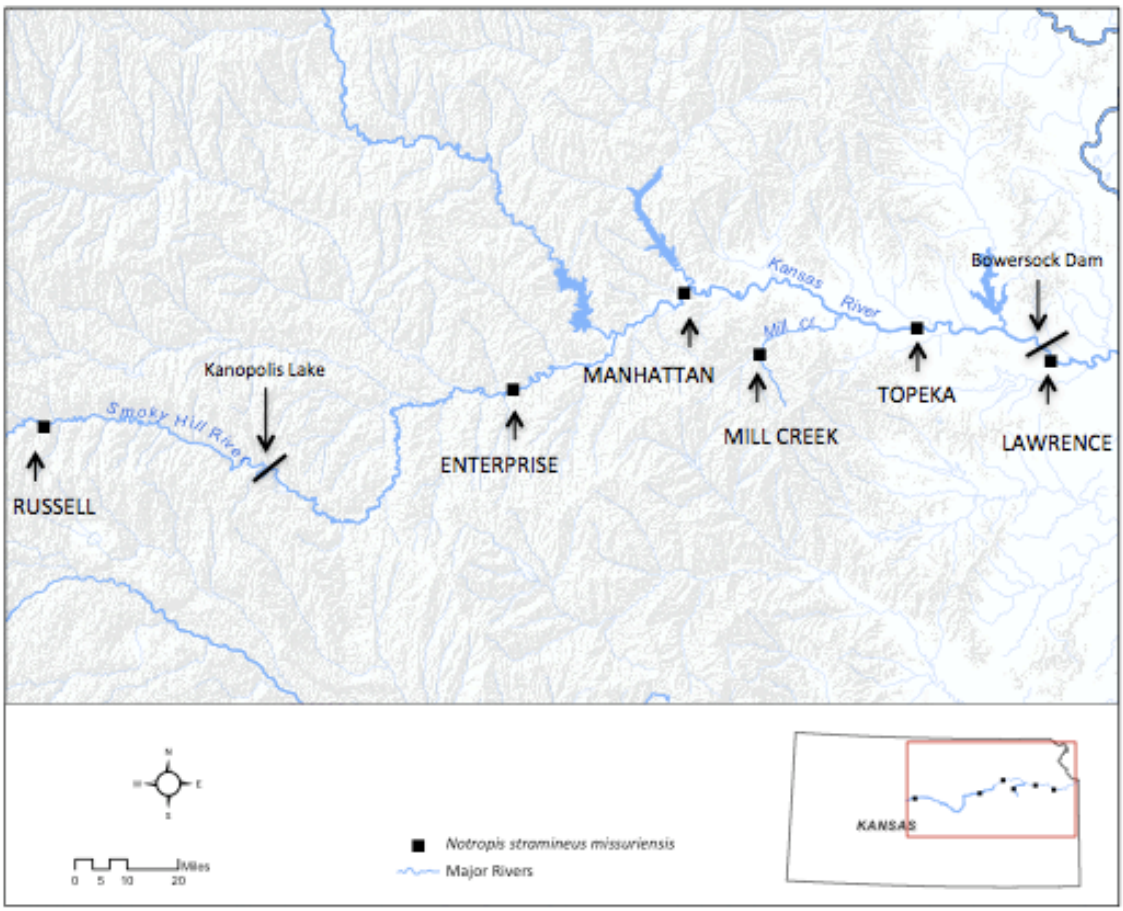

Figure 1
Map of collecting sites and pertinent barriers along the Kansas and Smoky Hill Rivers.

Introduction

Evaluating diversity within polytypic species often reveals unexpected cryptic diversity because speciation is not always accompanied by morphological change (reviewed in Bickford et al. 2007). In a polytypic species, diagnosable, or nearly diagnosable, geographic races are joined into a single species with each geographic race given a subspecies trinomial (Cracraft 1989). A common taxonomic practice was to give a separate species name to every differentiated population of a recognized species; thus, polytypic species were proposed in order to reduce the number of species names, and in 1948 the International Committee of Zoological Nomenclature formally recognized the taxonomic rank of subspecies (Wilson and Brown 1953). Naming subspecies was merely a "pigeon-holing" device for taxonomists (Mayr 1942, 1969); polytypic species designations, although useful to some taxonomists, do not necessarily reflect evolutionary processes or relationships (Cracraft 1989) and warrant further investigation.

Accurate species designations and accounts of diversity are central to all areas of biology (Mayr 1963, Bickford et al. 2007, Schlick-Steiner et al. 2010), thus biologists are left with the task of deciphering whether or not polytypic species contain structured genetic variation, and of deciding whether genetic variation correlates with described subspecies. Phylogeography provides an excellent set of tools for this undertaking. Phylogeographic analysis of genetic variation at, and below, the species level allows testing of hypotheses regarding past historical processes that can explain the current geographical distribution of genetic variation. Numerous phylogeographic investigations have tested the validity of subspecies as evolutionary units in a variety of taxa [e.g. fishes (Kreiser et al. 2001); lizards (Leache et al. 2010); snakes (Makowsky et al. 2010); birds (Manthey et al. 2011); mammals

(Edwards et al. 2011)]. In the present study, investigates the geographic structure of genetic variation in the polytypic North American minnow species *Notropis stramineus*.

Notropis stramineus (Cope), the Sand Shiner, is a common minnow found in clear, low gradient streams of all sizes that have cobble or sand substrate (Tanyolac 1973). The species has a widespread distribution in North America (Figure 1) ranging from the Rocky Mountains eastward to the western edges of Pennsylvania and New York State, and from the Great Lakes south to Tennessee. Two disjunct populations exist: one in southern Texas ranging from the Trinity River to the Rio Grande on the Gulf slope, and the other in the drainages of the Tennessee and Cumberland Rivers (Page and Burr 1991).

N. stramineus is currently divided into two subspecies (Tanyolac 1973) based on five morphological characters, the most distinctive being the number of circumference scales (the number of scale rows around the body immediately in front of the dorsal fin). The eastern subspecies, *N. stramineus stramineus*, has low circumference scale counts (22-25 scales) and occupies the majority of the range extending from Southern Saskatchewan to Quebec southward through the Great Lakes drainage and Ohio River basin; the Upper Mississippi basin west to Eastern Iowa, southern Missouri, and southeastern Kansas (including the Osage River System); the Red River drainage in Eastern Oklahoma and Texas, and southwestward in Gulf coast streams to the Rio Grande basin (Texas disjunct population). The western subspecies, *N. stramineus missuriensis*, has a high circumference scale count (27-30 scales) and occupies the Arkansas River and Missouri River drainages. Populations show intergrades of circumferential scale counts between the two subspecies in tributaries of the Missouri River, the Neosho River in Kansas, and the Upper Red River

System in western Oklahoma (Suttkus 1958, Bailey and Allum 1962, Tanyolac 1973) suggesting incomplete reproductive isolation between the subspecies.

Genetic variation in *N. stramineus* may not be structured according to subspecies designations but may instead reflect a speciation event resulting in a cryptic species complex with northern and southern clades. Using statistical parsimony and nested clade analysis, a population of *N. s. missuriensis* in the Kansas River is more closely related to populations *of N. s.stramineus* in South Dakota and Iowa than it is to a population of *N. stramineus missuriensis* in the Arkansas River (Michels 2000). Due to the small scale of the study, conclusions regarding a north-south split and the validity of *N. stramineus* subspecies could not be made.

In this study, I aimed to discover how genetic variation is structured across the distribution *N. stramineus* by using phylogeographic methods. I focused on two main objectives. First, I analyzed phylogenetic breaks in *N. stramineus* to determine if clades within this species correspond to the geographical distribution of subspecies. A pattern of reciprocal monophyly between the two subspecies, along with significant genetic differentiation, would suggest that a barrier to gene flow existed between the two subspecies. In a possible vicariance scenario, early deglaciation greatly widened the Missouri River through inputs of continental glacial meltwater. The Missouri River became so wide that it may have effectively formed a barrier to migration for *N. stramineus* populations on either side, and populations on the Western side of the Missouri River could have differentiated (e.g. through natural selection or genetic drift) while the western populations' access to gene flow from the eastern populations was severed. The Missouri River eventually narrowed after the supply of continental glacial meltwater to the Missouri

River was severed approximately 12,000 years ago (Clayton and Moran 1982, Teller 1987, Klassen 1994). This narrowing of the Missouri River would have reduced the hypothesized barrier to gene flow between eastern and western subspecies of *N. stramineus*, explaining why intergrades of the two purported subspecies of *N. stramineus* are found in areas around the Missouri River.

Alternatively, reciprocal monophyly and genetic differentiation between northern and southern populations would suggest that *N. stramineus* comprises a cryptic species complex with a distribution unrelated to currently described morphology. A north-south split could result from the vicariance events that formed the major pre-glacial streams. Three major pre-glacial stream are hypothesized by Metcalf (1966) to have drained the eastern United States before the Pleistocene: the southward flowing Teays/Mississippi system, the northward flowing Arctic/Hudson Bay system, and a pre-glacial Plains Stream system that flowed southward, mostly independent of the pre-glacial Teays/Mississippi system (Figure 2). Geological and faunal evidence have authenticated the existence of the Teays and Artic systems, though the westward extent of the Teays is still debated. The extent of Plains Stream has not been substantiated due to ambiguous geological evidence of its existence in Oklahoma (Cross et al. 1986). Metcalf (1966) hypothesized that the Plains Stream, or its glacial equivalent, the Ancient Plains Stream (APS), drained a large area of the Great Plains from Nebraska south of the ancestral Iowa River and west of the ancestral Grand River and continued south where it crossed the current Red River valley on its course to the Gulf of Mexico. The north-south split of the ancestral form of *N. stramineus* would have allowed the northern clade to diverge in the Teays system while the southern clade diverged in the southward flowing APS.

The second aim of this study is to address the origin of the disjunct distribution in south Texas by examining phylogenetic breaks and comparing genetic variation between the Texas populations and the rest of the distribution as a whole. Two likely origins for the Texas populations both involve range expansion followed by extinction of intervening populations. First, the Texas populations could have been established through various headwater captures of Rio Grande tributaries by Gulf Coast tributaries in southwest Texas. In this scenario, *N. stramineus* once had a continuous distribution in the Pecos River and southern Rio Grande River, with eventual extinction in the lower Pecos River. This hypothesis will be supported if the Texas populations are most closely related to populations in New Mexico. Secondly, the Texas disjunct population could have been established from the APS either directly or through headwater captures. If analyses show that Texas populations are most closely related to Plains populations (e.g. Red River) rather than New Mexico populations, a reasonable conclusion is that the Texas populations were established via the APS.

Materials and methods

Population sampling and DNA isolation

I acquired 231 specimens of *N. stramineus* from 18 sites (collected by myself and others during 1997-2009) across its known range (Fig. 1) with a primary focus on the Great Plains. Samples sizes ranged from 4 to 22 individuals per population. The specimens were preserved in either 95% ethanol or liquid nitrogen until DNA extraction was performed. I removed muscle tissue from the side of each specimen and extracted DNA using a DNeasy Tissue Kit (Qiagen). Extracted DNA was stored at -20 °C until needed. A list of specimens and complete locality and collection information are presented in Appendix 1 (Material Examined).

Laboratory Procedures

I amplified and sequenced an 812 bp fragment of the mitochondrial control region (D-loop) with the following primer pair: 5' AAGCATCGGTCTTGTAATCC 3' [16242-16261] and 5' TAGGACTCATCTTAGCATCTTCA 3' [959-970]. Positions in brackets are presented in relation to the sequence published for *Cyrprinus carpio* (Chang et al. 1994).

PCR was used to amplify the fragment in a 25 μL reaction containing ~ 10ng DNA in 1X buffer, 1.5 mM $MgCl_2$, 0.32 mM dNTP mix, 0.3μM of each primer, and 0.02 U of iTaq DNA Polymerase (Bio-Rad Corporation). The thermocycling protocol employed an initial denaturation at 94° C for 2 minutes followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 minute, and extension at 72°C for one minute with a final extension at 72°C for 5 minutes. PCR products were purified with either a QIAquick Purification Kit (Qiagen) or an ExoSAP-IT PCR Clean-up Kit (USB Corporation).

Purified DNA was sequenced by either ACGT, Inc. (Wheeling, IL) or the University of Kansas DNA Sequencing Laboratory (Lawrence, KS). Sequences were edited with Sequencher® (Gene Codes Corporation) and aligned using Clustal W (Larkin et al. 2007).

Seven microsatellite loci, previously screened for use in *N. stramineus*, were amplified using the same primers and PCR conditions as in Chapter 1. Samples were then multiplexed for fragment analysis by combining 5 µl of PCR product from 4 differently labeled microsatellites to form a pooled sample. The pooled samples were purified using Sephadex™ (GE Healthcare Life Sciences) and then sized by the University of Kansas DNA Sequencing Lab (Lawrence, KS). I manually genotyped fragments using Peak Scanner™ v1.0 (Applied Biosystems).

Phylogenetic Analyses

Mitochondrial DNA

In order to resolve more shallow relationships among mtDNA haplotypes, I used statistical parsimony to construct a mtDNA haplotype network with a 95% connection significance using TCS 1.21 (Clement et al. 2000). I employed traditional phylogenetic analyses to identify deep patterns of divergence among well-supported clades using both maximum likelihood (ML) and Bayesian inference (BI) approaches to construct gene trees with the collapsed haplotypes identified using TCS 1.21.

Both ML and BI gene tree estimation require the selection of an appropriate model of nucleotide evolution. I used jModelTest 0.1.1 (Posada 2008)

to evaluate 56 alternative substitution models. By applying the Akaike information criterion (AIC), I selected the most appropriate model of molecular evolution and used the parameters described by this model for all subsequent phylogenetic analyses.

ML analysis was performed using Garli 2.0 (Zwickl 2006), with four independent runs from random starting trees and using the auto-terminate setting and default parameters. I evaluated nodal support using 200 nonparametric bootstrap pseudoreplicates from which a 50% majority consensus tree was constructed from the pseudoreplicates in Mesquite 2.74 (Maddison and Maddison 2010). A BI analysis was executed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) by performing four independent runs each with four Metropolis-coupled Markov Chains with Monte Carlo simulation (MCMCMC) to guard against trapping of the analysis in local optima. Each run consisted of five million generations and sampled trees every 100th generation with a burn-in of 25% of the generations discarded at the conclusion of the runs. I combined samples from all runs to produce a 50% majority consensus tree using Mesquite. Both trees were rooted with *Cyprinella lutrensis* (Genbank accession number AB070206).

Microsatellite Loci

Microsatellite loci were evaluated for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium using the methods described in Chapter 1. I then used population alleles frequencies to estimate a neighbor-joining cladogram based on F^*_{ST} using POPTREE2 (Takezaki et al. 2010). I evaluated nodal support of the neighbor-joining tree using 1000 bootstrap replicates. Additionally, I used the Bayesian clustering analysis implemented by STRUCTURE 2.3.3 (Pritchard et al. 2000) to identify distinct population

groupings based on microsatellite alleles frequencies. While not a tree reconstruction, Bayesian cluster analysis does classify individuals into groups based on ancestry. STRUCTURE assumes a model with *K* populations, where each population is characterized by a set of allele frequencies at each locus. Individuals are then probabilistically assigned to populations or to more than one population if the genotypes indicates admixture. I tried different values of *K*, ranging from1 to 18, and ran STRUCTURE 20 times for 100,000 steps with a 50,000 step burn-in. I evaluated the correct value of *K* using the method of Evanno et al. (2005) and by visual inspection of the replicates. A value of K=1 would indicate no substructuring among populations while K=18 would indicate that each sampled population contains a unique set of allele frequencies.

Population Diversity and Structure

Summary statistics that express current genetic diversity of each sampled population were calculated using Arlequin 3.5.1.3 (Excoffier and Lischer 2010). For mtDNA haplotypes, I measured genetic diversity as the number of haplotypes, haplotype diversity (H_D), sequence diversity (K), and nucleotide diversity (π). For microsatellite loci, I measured genetic diversity as number of alleles per locus (A), observed heterozygosity (H_o), expected heterozygosity (H_E), and the inbreeding coefficient (F_{IS}).

Analysis of molecular variance (AMOVA; Excoffier et al. 1992), as implemented in Arlequin, was used to examine the geographic partitioning of genetic variation in *N. stramineus*. An AMOVA partitions genetic variation within and among user-specified groups. Variance components are then used to calculate analogs to Wright's F-statistics. I conducted AMOVA analyses separately for mtDNA and microsatellite alleles since

assumptions about their patterns of evolution differ. MtDNA AMOVAs were done by haplotype while the microsatellite AMOVAs were done on a locus-by-locus basis as described in Chapter 1. The significance of the fixation indices for each AMOVA was measured using 1000 permutations.

Two specific hypotheses were tested with the AMOVAs. First, I tested the hypothesis that genetic variation in *N. stramineus* is structured according to subspecies designations. Two separate AMOVAs, one using mtDNA and one using microsatellite allele frequencies, were used to test this hypothesis. In this analysis, collection localities were treated as "populations" and subspecies were treated as "groups." Second, I investigated the significance of genetic variation within and among the major clades identified by the phylogenetic analyses of mtDNA. A single AMOVA using microsatellite allele frequencies was used to test this hypothesis since the clades were hypothesized using mtDNA. In this analysis, collection localities were treated as "populations" and the clades were treated as "groups."

RESULTS

Phylogenetic analyses

MtDNA

Statistical parsimony identified 97 unique haplotypes (Appendix 1) and produced 4 unconnected minimum spanning networks, each containing haplotypes connected at the 95% confidence level (Figure 3). If statistical parsimony cannot connect haplotypes without assuming multiple mutations, multiple networks will result. The four networks correspond to the following drainages: (1) Missouri River, Upper Mississippi River, and Lower Mississippi; (2) Arkansas River, Canadian River, Pecos River; (3) Red River and Ohio River; and (4) Texas Gulf. The complete data set of haplotype sequences is given in Appendix 3.

JModelTest identified GTR + I + Γ (generalized time reversible model with a proportion of invariable sites and gamma distributed rate heterogeneity) as the best-fitting evolutionary model for the data. Using this model, ML and BI analyses both recovered similar topologies. The ML tree has 5 well-supported clades (BS > 0.75): (Clade M) Missouri River, Upper Mississippi River, and Lower Mississippi River; (Clade A) Arkansas River, Canadian River, and Pecos River; (Clade R) Red River; (Clade T) Texas Gulf; and Clade (O) Ohio River (Figure 4). Though well supported, the clades are not well structured with respect to each other and basal relationships cannot be determined. The BI tree recovers four major clades (which are the same groups found by the minimum spanning network) with structuring but the posterior probabilities of many of these branches are not high (PP<0.95) (Figure 5). If one ignores the non-significant branches, 5 major clades are recovered, which are the same clades identified in the ML tree.

Microsatellites

Microsatellite locus 4 repeatedly failed to amplify in many specimens from the Arkansas and Canadian Rivers. This is highly suggestive of the presence of null alleles in these populations so Locus 4 was discarded from further analyses. The complete data set of microsatellite genotypes is given in Appendix 4.

Eight pairs of loci exhibited linkage disequilibrium in nine populations (α=0.05, $P < 0.05$) but only two of the comparisons were significant following Bonferroni correction (α=0.05, $P < 0.0002$; data not shown). Both of these pairs involved loci that had only 2 or 3 alleles with one locus near fixation for a single allele. Because neither pair of loci appeared linked in any other populations, they were retained for analysis as independent loci.

Tests of deviation from Hardy-Weinberg equilibrium are presented in Table 1. Eighteen population-locus combinations showed significant deviation from Hardy-Weinberg equilibrium (α=0.05, $P < 0.05$) but following Bonferroni correction, only one population-loci combination (WV, locus 5) showed deviation from Hardy-Weinberg equilibrium (α=0.05, $P < 0.0005$). Examination of this locus in other populations does not indicate the presence of null alleles. Additionally, removal of this locus did not markedly affect the outcomes of further analyses. Thus, the locus was retained for further analysis.

The inbreeding coefficient F_{IS} was significantly different from zero (α=0.05, $P < 0.05$) in 10 populations before Bonferroni correction, with all values being positive. Following correction, only two populations (KS-1 and KS-2) exhibited a significant deviation from zero (α=0.05, $P < 0.003$). A significant, positive F_{IS} indicates heterozygote deficiency, possibly caused by inbreeding. Heterozygote deficiency is a common finding in fishes and may be an artifact of sampling (see Chapter 1 for discussion).

The NJ tree identified the same five clades as the mtDNA phylogenies with one major difference (Figure 6). While mtDNA places the KY population in Clade M (Missouri and Mississippi River drainages), microsatellites place the KY population within Clade A (Arkansas River drainages).

The STRUCTURE analysis returned *K*=2 as the most likely number of clusters for the microsatellite data. One cluster consists of Texas Gulf coast, Red River populations while the other cluster contains all other populations. The analysis ended up being confounded by two particular loci. In the Texas disjunct and Red river populations, alleles at these loci were fixed, or nearly fixed. Additionally, these alleles were rare in other populations. This caused STRUCTURE to always group these populations together in a cluster. No matter how many clusters were modeled, the Texas and Red River populations always grouped together in a cluster with greater than 98% identity.

Population diversity and structure

The summary of genetic diversity measures for mtDNA and microsatellite are presented in Tables 1 and 2. Haplotype diversity (H_D) was moderately to very high (H_D >0.65) in most populations but was notably lower ($H_D < 0.54$) $_{in}$ the RD-1, RD-2, WV and NM populations. Sequence and nucleotide diversity (K AND π) was highest in KY, IA, CA-1, CA-2, and NM populations while they were lowest in SD, RD-1, RD-2, and WV populations.

Microsatellite allelic diversity, measured as average observed heterozygosity (H_O) diversity, was lowest in the TX-1 and TX-2 populations. TX-1 has 2 fixed loci (Loci 6 and 7) with two more loci nearly fixed (Loci 2 and 4). TX-2 has three fixed loci (Loci 2, 6, and 7) and one locus nearing fixation (Locus 3) but it should be noted that TX-2 has the smallest

sample size (n=5). Interestingly, these populations still retain several mitochondrial haplotypes.

Analysis of molecular variance using mtDNA haplotypes refutes the hypothesis of genetic structure between the two described subspecies of *N. stramineus*. If the two subspecies of *N. stramineus* were genetically divergent, the expectation would be that an AMOVA with the populations grouped by subspecies would be significant. Additionally, there should be more variance among the subspecies than among the populations within the subspecies. The mtDNA AMOVA (Table 3) with populations grouped according to subspecies was non-significant (F_{CT} = 0.04190, P=0.21799), and the vast majority of the variation in this hierarchy was among populations within subspecies (80.83%) with little variation among the subspecies (4.19%).

Analyses of molecular variance using microsatellite allele frequencies also support the hypothesis of genetic structure between five clades though both subspecies (Table 4) and five clade (Table 5) analyses were statistically significant (subspecies F_{CT}=0.05728, P=0.00098; five clades F_{CT} = 0.15710, P<0.00000).

A statistically significant AMOVA indicates that F_{CT} is greater than 0 and that there is variation among groups but this does not mean that the variation is biologically meaningful. In order to selecting the best competing hypothesis , comparison of F_{CT} values from each AMOVA is necessary. The amount of variation among groups in the five clades AMOVA (15.71%) is almost triple the variation among groups in the subspecies AMOVA (5.73%). Additionally, variation among populations within groups in the five clades AMOVA (8.62%) is less than half the variation among populations in the subspecies AMOVA (17.31%)

indicating that populations within each clade are far more alike than populations within each subspecies.

Discussion

Identification of cryptic species

Analysis of both mtDNA and nuclear microsatellites refute the current subspecies designations of *N. stramineus* as valid evolutionary lineages. The evidence instead supports the recognition of a cryptic species complex composed of at least 5 species. This finding is bolstered by concordance between mitochondrial and nuclear markers.

Five clades are recovered by phylogenetic analyses using both mtDNA and microsatellites. The only discordance found between gene trees was for the KY population in the Lower Mississippi River drainage. This population has Clade M mtDNA and Clade A nuclear DNA. Discordance between gene trees can be caused by incomplete lineage sorting, retention of ancestral polymorphisms, hybridization, or introgression. Biased mitochondrial introgression is not uncommon in animal lineages and has been has been documented in several North American fishes including minnows (Berendzen et al. 2008), darters (Bossu and Near 2009), and char (Doiron et al. 2002). The bias can be caused by an array of different mechanisms, either acting independently or in combination, and include cytonuclear compatibilities (Rieseberg et al. 1996a), demographic effects (Rieseberg et al. 1996b), sexual selection and asymmetric reproductive barriers (Chan and Levin 2005), and differences in the magnitude of selection on certain genes (Funk and Omland 2003).

The individuals in the KY population appear to maintain a Clade A nuclear background as evidenced by microsatellite allele frequencies. Additionally, the mtDNA in this population is most closely related to the nearest sampled population (IA) of Clade M individuals. Further sampling of populations along the Mississippi River, and the addition of nuclear sequences, may show further incidences of introgression. Recovery of the KY

population as a member of Clade A indicates that Clade A may once have had a more contiguous distribution with populations in the Interior Highlands of southern Missouri.

The introgression of Clade M mtDNA into the Clade A genome most likely occurred via downstream migration of Clade M individuals. The Mississippi River poses a general barrier to eastern and western populations of the *N. stramineus* species complex as it contains very little suitable habitat for these species. Individuals of *N. stramineus* are rarely found in the mainstem of the Mississippi River, and mostly likely, there are not any permanent populations in this river (Bailey and Allum 1962). The occasional presence of *N. stramineus* does indicate that it is able to use the river for dispersal.

Distribution and diversity of lineages

Due to a lack of samples east of the Mississippi River, the eastern extent of species distributions cannot be ascertained from this study but general geographical breaks can be identified (Figure 7).

Clade M contains populations in the Missouri River and Western Mississippi River drainages. This clade may extend east of the Mississippi River until it meets the distribution of Clade O or the distribution of Clade O could extend westward to the Mississippi River. Populations in Clade M show very high haplotypic diversity with the lowest diversity in the SD population. The SD population also has the lowest nucleotidic diversity in this clade. This suggests that SD might be the youngest population from Clade M sampled in this study.

Clade A contains population from the Arkansas and Canadian Rivers, a disjunct population in the Pecos River drainage, and a disjunct population in the Lower Mississippi River drainage. The contiguous distribution in the Arkansas and Canadian Rivers extends

from the mouths of the rivers eastward until the Arkansas River meets the Interior Highlands in southern Missouri and western Arkansas. The disjunct population in Kentucky may possibly extend further north along the Mississippi River or east along the Ohio River where it meets the distribution of Clades M and O, respectively.

Clade R contains populations from the Red River and its tributaries. The distribution extends east until the Red River makes an abrupt southern turn as it enters the Interior Highlands of southern Arkansas. It does not extend all the way to the Mississippi River. This results in the clade being effectively disjunct as it has no gene flow with other clades.

Clade T contains small populations from numerous gulf coast drainages (Colorado, Brazos, Nueces, San Antonio Bay, and Galveston Bay) in the Edwards Plateau of Texas. While populations in this clade have moderate levels of haplotypic diversity, allelic diversity is quite low. Low allelic diversity could indicate either a small founding population or a recent population bottleneck, which are both plausible, but because mtDNA diversity is not correspondingly low, this could just be an anomaly. Further investigations will be necessary to address historical population size in this clade.

In this study, Clade O is represented by only one population so it is not possible to know the extent of its distribution. It is reasonable to expect that it occupies the Ohio River drainages in Northern Kentucky and southern Ohio. Clade O meets Clades M and A somewhere in the region where the Ohio River watershed meets with the Upper Mississippi River watershed.

Historical Biogeography and ancient drainage patterns

Identification of cryptic diversity within *N. stramineus* and an understanding of evolutionary relationships among the newly revealed clades can provide insight into the geographical events (e.g. ancient and modern drainage patterns) that shaped divergence in this species complex. Snelson (1971) proposed that *N. stramineus* originated from a *N. stramineus*-like ancestor that had a wide distribution across eastern North America. This ancestor diverged to produce the *Notropis procne* species complex in which *N. stramineus* and *N. topeka* are the only taxa outside of the northern Central Highlands. Starting with a widespread ancestral *N. stramineus*, a general biogeographic history of the *N. stramineus* species complex can be postulated.

It is likely that divergence in the *N. stramineus* species complex originated in the Great Plains. The first known fossil of *N. stramineus* was found in southwest Kansas, and it dates to the pre-Pliocene (Cross et al. 1986). Teays populations (Clade M) possibly became separated from Plains populations by formation of the APS during the Pliocene. During the Pleistocene glaciations, Clade M would have retracted to the south and west and then repopulated drainages back to the north and east once the final glaciers retracted. The current distribution of Clade M could have been established as the APS dissolved and the northernmost APS drainages were diverted to the modern day Missouri River Basin. The current Missouri River Basin was formed as the "Kansan" glacier retreated allowing the Kansas River to flow east. The Smoky Hill River, a tributary of the Kansas River, was transferred from the Arkansas River drainage to the Kansas River by the time of the Wisconsinian glacier (Cross et al. 1986). In this scenario, Clade M would have successfully recolonized the Kansas River drainage by out-competing the remaining Clade R individuals.

Plains populations may have diverged during the Pleistocene to become Clades A and R as the Arkansas and Red Rivers separated from the Ancestral Plains Stream. Clade A then expanded into the Pecos River through stream capture. The low haplotypic diversity of the NM population and the close proximity of the headwaters of the Pecos and Canadian Rivers suggest that this expansion into the Pecos River is recent and occurred via a stream capture of a Canadian River tributary in northeast New Mexico. This supports the hypothesis of Conner and Suttkus (1986) that the Pecos River obtained its current drainage basin by capturing the headwaters of other rivers, including tributaries of the Canadian River, during the late Pleistocene.

If the APS did run all the way to the Gulf, Clade T likely evolved as the APS disintegrated and gulf coast drainages acquired their modern pathways to the Gulf of Mexico. Because no conclusive evidence exists that the APS ran all the way to the gulf (Cross et al. 1986), alternate hypotheses need to be considered. The STRUCTURE analysis of microsatellites indicates that Clade T and Clade R are closely related and may have shared a common bottleneck event following their founding. Clade T could have been founded by migrants from the ancestral Red River via stream capture in northwest Texas. Indirect geologic evidence (e.g. diversion elbows and regions of abrupt directional change) indicates that the upper reaches of the Brazos and Colorado Rivers were derived from stream captures of some unknown southern Great Plains river system such as the ancestral Red River (Connor and Suttkus 1986). Several other Plains fishes have a western Gulf component to their distributions and may have also been derived via ancestral Red River stream captures. These include *Fundulus zebrinus*, *Cyprinella lutrensis*, *Hybognathus placitus*, and *Phenacobius mirabilis* (Kreiser et al. 2001).

Further study is needed to construct a biogeographical history of Clade O. It should be noted that in this study Clade O shows a slight affinity for Clade R in the minimum spanning network, NJ tree, and BI tree, indicating some shared ancestry (possibly from a largely distributed southern ancestor) but the close relatedness of Clades O and R could also simply be an anomaly (Wiley 2011, personal communication). I defer speculating on this affinity until more data is collected from additional eastern populations.

Conclusion and future directions

Mitochondrial and nuclear markers reject the validity of *N. stramineus* subspecies and suggest the presence of 5 cryptic species in the geographical area studied. Divergence between these clades was facilitated by extensive alterations of stream drainages during glaciations and the formation of modern watersheds. Morphological difference between subspecies, particularly scale counts, may merely reflect phenotypic plasticity invoked by an environmental cline. Numerous external and internal environmental factors have been shown to influence the number of scales developed during ontology, including temperature, salinity, pollution, parasitism, and alterations in thyroid hormone levels (see review in Levin 2011).

Subspecfic variation aside, the clades of the *N. stramineus* species complex share a common morphology. If diversification was recent, it could be the case that not enough time has passed for the morphology of these clades to diverge. Alternatively, common morphology could be due to selective pressures of the similar environments inhabited by these clades resulting in shared morphological characters. Additional study is needed to

identify possible morphological differences between clades, and I address this topic in Chapter 3.

Finally, it is possible that more species exist in this complex in the unsampled areas east of the Mississippi River. Numerous cryptic clades have been found in the *Etheostoma spectabile* species complex that shares a similar widespread distribution with the *N. stramineus* species complex (Ceas and Page 1997, Ceas and Burr 2002, Lang and Mayden 2007, Bossu and Near 2009). Particular attention should be paid to populations in the Tennessee-Cumberland Rivers and Great Lakes watersheds. Addition of nuclear gene sequences may provide a more robust phylogeny and could identify additional instances of mitochondrial introgression. Future investigations of these populations will be able to provide additional insight into the evolution of the *N. stramineus* species complex.

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Table 1
Individual microsatellite locus statistics for *Notropis stramineus* populations. Bold values are statistically significant ($p \leq 0.05$) before Bonferonni correction. Asterisks indicate values that were statistically significant following Bonferonni correction for simultaneous tests.

Population	Par.	Locus 1	Locus 2	Locus 3	Locus 5	Locus 6	Locus 7	Average
KY	A	2	8	9	7	2	4	5.330
(N=18)	H_O	0.00000	0.94444	0.77778	0.55556	0.50000	0.83333	0.60185
	H_E	0.10794	0.80317	0.83333	0.66667	0.50000	0.74127	0.60873
	P_{HW}	**0.02837**	0.64198	0.30702	0.15781	1.00000	0.52046	---
	F_{IS}	1.00000	-0.18200	0.06849	0.17073	0.00000	-0.12832	0.01163
KS-1	A	7	16	12	9	4	2	8.333
(N=19)	H_O	0.41176	0.94737	0.72222	0.57895	0.36842	0.05263	0.51356
	H_E	0.65241	0.93314	0.88413	0.82788	0.60740	0.05263	0.65960
	P_{HW}	**0.03176**	0.79891	0.07433	**0.04557**	**0.00310**	1.00000	---
	F_{IS}	0.37604	-0.01567	0.18750	0.30648	0.40000	0.00000	**0.20093***
AR-1	A	4	10	8	5	3	3	5.500
(N=8)	H_O	0.50000	1.00000	0.50000	0.37500	0.62500	0.75000	0.62500
	H_E	0.57500	0.91667	0.90833	0.60000	0.69167	0.69167	0.73056
	P_{HW}	1.00000	1.00000	**0.00917**	0.13213	0.70133	**0.47699**	---
	F_{IS}	0.13846	-0.09804	0.46667	0.39130	0.10256	-0.09091	**0.15323**
AR-2	A	3	11	12	10	5	4	7.50000
(N=13)	H_O	0.30769	0.84615	0.69231	0.69231	0.61538	0.76923	0.65385
	H_E	0.46462	0.89846	0.92308	0.81231	0.74154	0.70154	0.75692
	P_{HW}	0.42045	0.19703	0.07574	0.19568	0.22212	0.75231	---
	F_{IS}	0.34694	0.06050	0.25773	0.15294	0.17597	0.17597	**0.14105**
WV	A	3	6	9	6	5	2	5.16700
(N=19)	H_O	0.52632	0.68421	0.73684	0.21053	0.36842	0.63158	0.52632
	H_E	0.52489	0.80228	0.76102	0.77952	0.43243	0.51209	0.63537
	P_{HW}	0.09890	**0.00515**	0.57019	**0.00000***	0.38460	0.37884	---
	F_{IS}	-0.00279	0.15064	0.03263	0.73529	0.15152	-0.24138	**0.17557**
RD-1	A	2	2	6	6	1	1	3.00000
(N=10)	H_O	0.10000	0.60000	0.44444	0.50000	0.00000	0.00000	0.41111
	H_E	0.39474	0.50526	0.78431	0.78947	0.00000	0.00000	0.61845
	P_{HW}	0.04619	1.00000	**0.00851**	0.10155	---	---	---
	F_{IS}	0.75676	-0.20000	0.44828	0.37931	---	---	**0.32235**
TX-2	A	3	1	2	3	1	1	1.83300
(N=5)	H_O	0.60000	0.00000	0.20000	0.40000	0.00000	0.00000	0.40000
	H_E	0.73333	0.00000	0.55556	0.51111	0.00000	0.00000	0.60000
	P_{HW}	0.54034	---	0.36598	0.33104	---	---	---
	F_{IS}	0.20000	---	0.66667	0.23810	---	---	0.36000
TX-1	A	3	2	7	2	1	1	2.667
(N=11)	H_O	0.63636	0.00000	0.72727	0.18182	0.00000	0.00000	0.38636
	H_E	0.58442	0.17316	0.83550	0.17316	0.00000	0.00000	0.44156
	P_{HW}	0.47769	0.04803	0.12777	1.00000	---	---	
	F_{IS}	-0.09375	1.00000	0.13514	-0.05263	---	---	0.13043

Table 1 continued

Population	Par.	Locus 1	Locus 2	Locus 3	Locus 5	Locus 6	Locus 7	Average
RD-2	H_O	0.00000	0.54545	0.45455	0.63636	0.00000	0.00000	0.54545
(N=11)	H_E	0.00000	0.56710	0.82251	0.79221	0.00000	0.00000	0.72727
	P_{HW}	---	1.00000	**0.01014**	0.55784	---	---	---
	F_{IS}	---	0.04000	0.45946	0.20455	---	---	**0.25926**
NM	A	4	8	7	7	4	4	5.667
(N=19)	H_O	0.36842	0.68421	0.89474	0.63158	0.73684	0.57895	0.64912
	H_E	0.73400	0.81223	0.77240	0.79659	0.64723	0.62020	0.73044
	P_{HW}	**0.00481**	0.09625	0.87029	0.05432	0.61845	0.69466	---
	F_{IS}	0.50491	0.16129	-0.16350	0.21168	-0.14286	0.06824	**0.11407**
CA-1	A	4	10	9	11	3	4	6.833
(N=11)	H_O	0.54545	0.90909	0.81818	0.72727	0.63636	0.63636	0.71212
	H_E	0.59307	0.89177	0.87013	0.91342	0.58442	0.70563	0.75974
	P_{HW}	0.60423	0.70967	0.89289	0.1659	0.61732	0.81457	---
	F_{IS}	0.08397	-0.02041	0.0625	0.21182	-0.09375	0.10256	0.06561
CA-2	A	4	14	9	8	4	5	7.333
(N=15)	H_O	0.53333	0.93333	0.60000	0.66667	0.53333	0.60000	0.64444
	H_E	0.56092	0.90805	0.86437	0.85287	0.74483	0.64138	0.76207
	P_{HW}	0.84849	0.54134	**0.00391**	0.11971	0.10432	0.80695	---
	F_{IS}	0.05085	-0.02887	0.31335	0.22438	0.29114	0.06667	**0.15898**
IA	A	2	8	7	9	2	2	5
(N=11)	H_O	0.10000	0.80000	0.54545	0.90909	0.54545	0.36364	0.54394
	H_E	0.10000	0.82105	0.83550	0.84416	0.41558	0.48485	0.58352
	P_{HW}	1.00000	0.85551	**0.03354**	0.73201	0.50498	0.53619	---
	F_{IS}	0.00000	0.02703	0.35829	-0.08108	-0.33333	0.25926	0.04891
PL-2	A	2	9	6	4	2	1	4.600
(N=20)	H_O	0.36842	0.80000	0.55000	0.45000	0.30000	0.00000	0.49368
	H_E	0.51351	0.79359	0.63590	0.51538	0.38462	0.00000	0.5686
	P_{HW}	0.35619	0.56206	0.68120	0.83790	0.54315	---	---
	F_{IS}	0.28814	-0.00829	0.13814	0.12977	0.22449	---	0.12541
SD	A	3	9	6	7	2	3	5.000
(N=19)	H_O	0.52632	0.84211	0.36842	0.57895	0.42105	0.10526	0.47368
	H_E	0.59175	0.85917	0.52205	0.83642	0.44381	0.10384	0.55951
	P_{HW}	0.20574	0.83299	**0.04162**	**0.00062**	1.00000	1.00000	---
	F_{IS}	0.11330	0.02041	0.30000	0.31369	0.05263	-0.01408	**0.15698**

Table 1 continued

Population	Par.	Locus 1	Locus 2	Locus 3	Locus 5	Locus 6	Locus 7	Average
NI	A	2	6	6	3	2	1	3.333
(N=9)	H_O	0.50000	1.00000	0.88889	0.33333	0.22222	0.00000	0.58889
	H_E	0.50000	0.79739	0.83660	0.46405	0.20915	0.00000	0.56144
	P_{HW}	1.00000	0.41502	0.94715	0.24938	1.00000	---	---
	F_{IS}	0	-0.27434	-0.06667	0.29412	-0.06667	---	-0.07494
PL-1	A	2	7	5	7	1	2	4.000
(N=10)	H_O	0.62500	0.80000	0.70000	0.50000	0.00000	0.20000	0.56500
	H_E	0.52500	0.72632	0.74737	0.77895	0.00000	0.18947	0.59342
	P_{HW}	1.00000	0.71226	0.00867	**0.02344**	---	1.00000	---
	F_{IS}	-0.20690	-0.10769	0.06667	0.37063	---	-0.05882	0.028
KS-2	A	5	15	11	7	4	4	7.667
(N=10)	H_O	0.47368	0.84211	0.63158	0.47368	0.47368	0.15789	0.50877
	H_E	0.52916	0.92888	0.86486	0.78663	0.51778	0.15363	0.63016
	P_{HW}	0.32416	0.58908	**0.00774**	**0.00169**	0.55331	1.00000	---
	F_{IS}	0.10744	0.09576	0.27517	0.40441	0.08732	-0.02857	**0.19692***
Avg. F_{IS}		0.21076	0.0132	0.18788	0.28359	0.10356	-0.02424	

N – number of individuals in the sample; A – observed number of alleles per locus; H_o – observed heterozygosity; H_E – expected heterozygosity; P_{HW} – probability of departure from Hardy-Weinberg equilibrium; F_{IS} – inbreeding coefficient.

Table 2
Molecular diversity values for mtDNA.

Population	Abbreviation & Sample Code	N_S	N_H	S	H_d	K	π
Mayfield Creek, KY	KY (B)	20	6	25	0.8579 +/- 0.0363	9.168421 +/- 4.402055	0.011319 +/- 0.00607
Cedar Creek, IA	IA (Y)	11	11	24	1.0000 +/- 0.0388	5.818182 +/- 3.013703	0.007183 +/- 0.004197
Six Mile Creek, SD	SD (AA)	13	4	3	0.7308 +/- 0.0879	0.923077 +/- 0.679572	0.001142 +/- 0.000945
Niobrara River, NE	NI (BB)	8	8	9	1.0000 +/- 0.0625	3.071429 +/- 1.782724	0.003801 +/- 0.002513
East Platte River, NE	PL-1 (CC)	10	8	11	0.9333 +/- 0.0773	2.355556 +/- 1.400264	0.002908 +/- 0.001955
West Platte River, NE	PL-2 (Z)	20	11	10	0.8053 +/- 0.0903	2.084211 +/- 1.215337	0.002579 +/- 0.00168
East Kansas River, KS	KS-1 (D,X)	19	15	14	0.9708 +/- 0.0273	2.116959 +/- 1.23323	0.002620 +/- 0.001706
Smoky Hill River, KS	KS-2 (EE)	17	12	11	0.9191 +/- 0.0571	1.911765 +/- 1.144158	0.002366 +/- 0.001585
Slate Creek, KS	AR-1 (E)	7	5	7	0.9048 +/- 0.1033	3.333333 +/- 1.942267	0.004131 +/- 0.002757
Arkansas River, KS	AR-2 (LL)	12	9	11	0.9545 +/- 0.0467	4.500000 +/- 2.38208	0.005576 +/- 0.003323
Sand Creek, OK	CA-1 (S)	11	5	18	0.8182 +/- 0.0826	6.854545 +/- 3.496063	0.008494 +/- 0.004887
Turkey Creek, OK	CA-2 (T)	14	6	20	0.7692 +/- 0.0895	8.043956 +/- 3.976196	0.009968 +/- 0.005531
Pecos River, NM	NM (R)	18	3	18	0.3856 +/- 0.1280	5.326797 +/- 2.696617	0.006601 +/- 0.003737

Table 2 continued

Population	Abbreviation & Sample Code	N_S	N_H	S	H_d	K	π
Pennington Creek, OK	RD-1 (L)	10	4	4	0.5333 +/- 0.1801	1.111111 +/- 0.789457	0.001377 +/- 0.001106
Bois D'Arc Creek, OK	RD-2 (Q)	9	2	2	0.5000 +/- 0.1283	1.000000 +/- 0.739434	0.001239 +/- 0.001039
Johnson Creek, TX	TX-1 (P)	9	6	8	0.8889 +/- 0.0910	2.833333 +/- 1.645489	0.003507 +/- 0.00231
Nueces, TX	TX-2 (N)	4	2	5	0.6667 +/- 0.2041	3.333333 +/- 2.148861	0.004131 +/- 0.00318
Sandy River, WV	WV (H)	19	5	9	0.5263 +/- 0.1266	1.122807 +/- 0.764335	0.001391 +/- 0.001058

N_S = Number of individuals sampled in the population, N_H= number of haplotypes identified in the population, S= number of polymorphic sites, H_d= Haplotypic diversity, K= sequence diversity, and π = nucleotide diversity. Abbreviation refers to population names used in text. Sample Code refers to taxa names of haplotypes at tips of trees in phylogenetic analyses.

Table 3
AMOVA design and results using mtDNA with populations grouped according to subspecies.

Source of Variation	d.f.	Sum of squares	Variance components	Percentage of variation	F	P
Among Groups	1	210.336	0.52446 Va	4.19	0.04190	0.21799
Among Populations within Groups	16	2071.344	10.11765 Vb	80.83	0.84370	0.00000
Within Populations	213	399.251	1.87442 Vc	14.98	0.85024	0.00000
Total	230	2680.931	12.51653			

Table 4
Global AMOVA design and results using microsatellite loci with populations grouped according to subspecies

Source of Variation	Sum of squares	Variance components	Percentage of variation	F	P
Among Groups	46.655	0.13183	5.27	0.05728	0.00098
Among Populations within Groups	204.674	0.39853	17.32	0.18367	0.00000
Among Individuals within Populations	467.216	0.28414	12.35	0.16042	0.00000
Within Individuals	365	1.48709	64.61	0.35839	0.00000
Total	1083.545	2.30159			

Table 5
Global AMOVA design and results using microsatellite loci with populations grouped according to subspecies.

Source of Variation	Sum of squares	Variance components	Percentage of variation	F	P
Among Groups	46.655	0.13183	5.27	0.05728	0.00098
Among Populations within Groups	204.674	0.39853	17.32	0.18367	0.00000
Among Individuals within Populations	467.216	0.28414	12.35	0.16042	0.00000
Within Individuals	365.00	1.48709	64.61	0.35839	0.00000
Total	1083.545	2.30159			

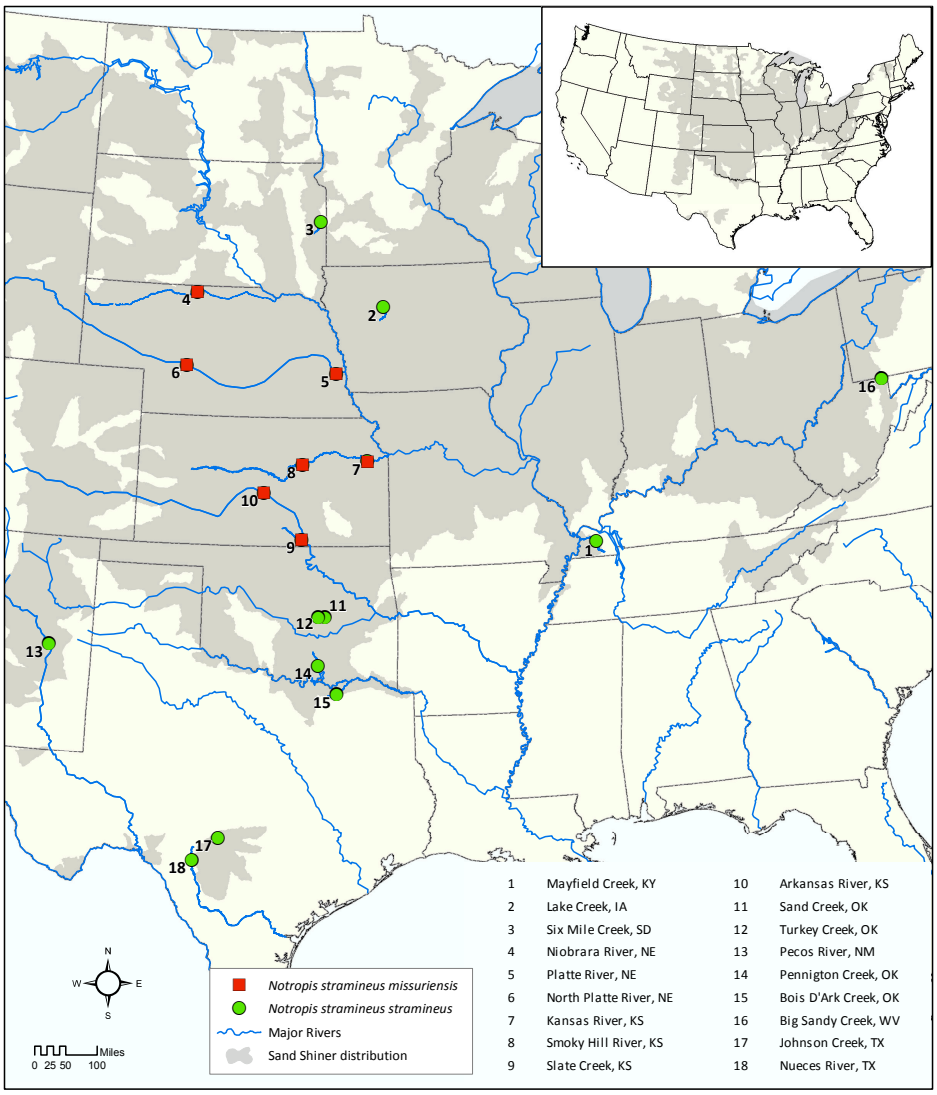

Figure 1
Distribution of *Notropis stramineus* (brown shading) and location of sampled populations used in this study. Green circles and red squares represent eastern and western subspecies, respectively.

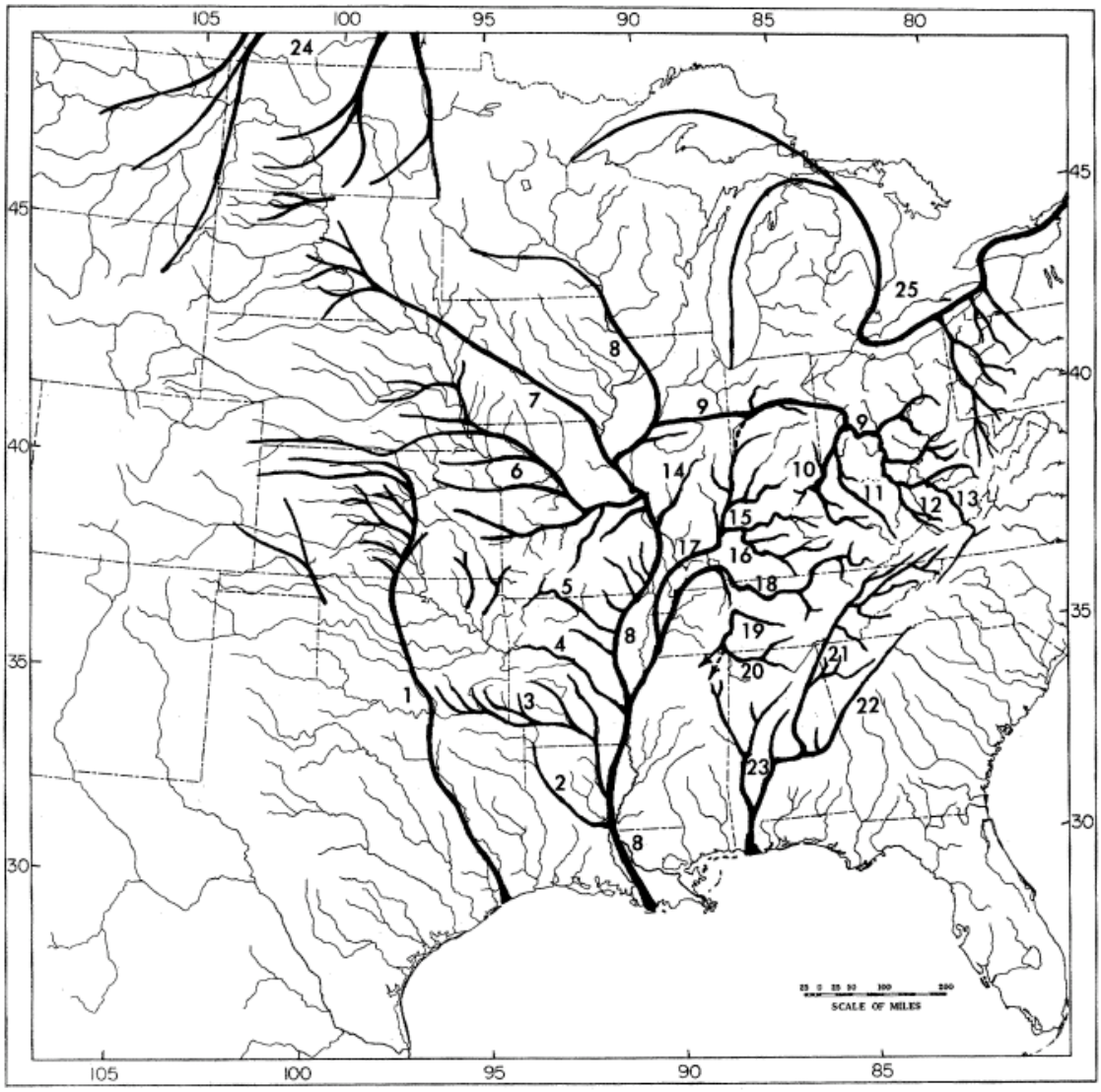

Figure 2
Reconstructed North American preglacial drainages overlaid on current drainage patterns (Mayden 1988). 1-Plains Stream. 2-Old Red River. 3-Old Ouachita River. 4-Old Arkansas River. 5-White River. 6-Old Grand-Missouri River. 7-Ancestral Iowa River. 8-Old Mississippi River. 9-Old Teays-Mahomet River. 10-Old Kentucky River. 11-Old Licking River. 12-Old Big Sandy River. 13-Kanawha River. 14-Kaskaskia River. 15-Wabash River. 16-Green River. 17-Old Ohio River. 18- Old Cumberland River. 19-Old Duck River. 20-Old Tennessee River. 21-Appalachian River. 22-Old Tallapoosa River. 23-Mobile Basin. 24-Hudson Bay Drainage. 25-St. Lawrence River.

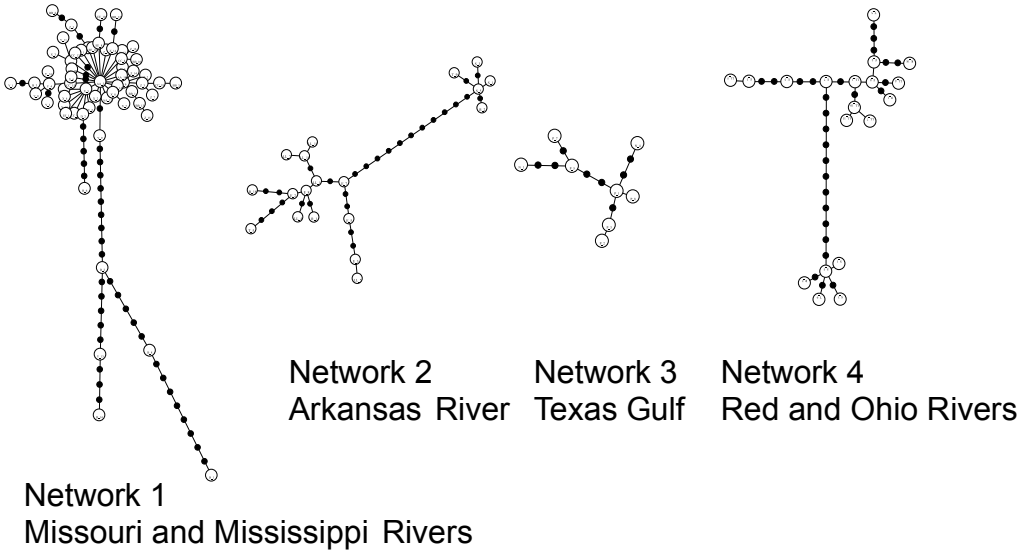

Figure 3
The four unconnected minimum spanning networks from statistical parsimony calculated with 95% connection significance. Each open circle represents a unique haplotype. Each line connecting two haplotypes represents a single mutation. Each dark circle represented a mutational step (missing haplotype).

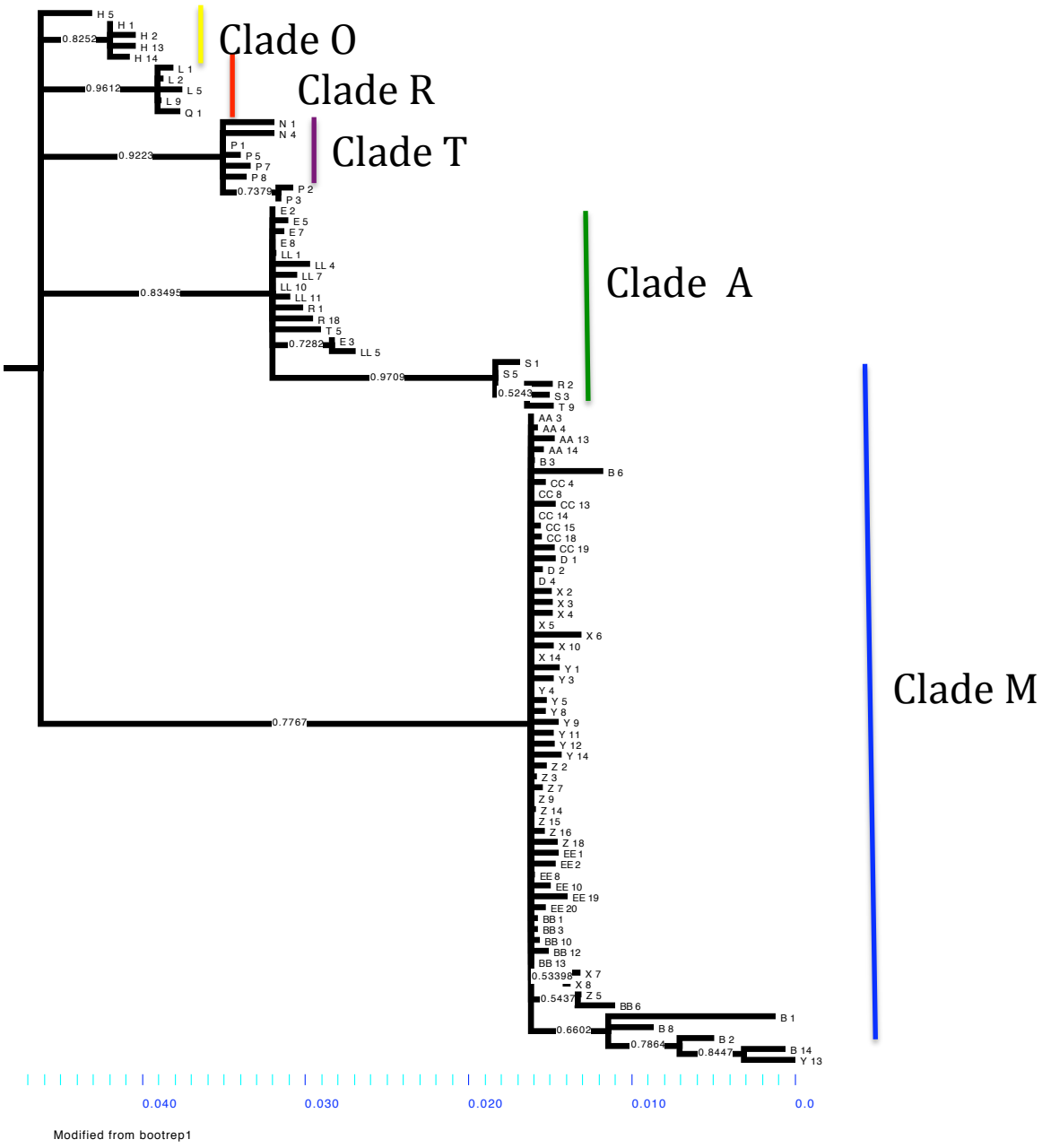

Figure 4
Mitochondrial D-loop maximum likelihood phylogeny computed with 200 bootstrap pseudoreplicates. Numbers on branches indicate bootstrap values. The tree was rooted with *Cyprinella lutrensis*. The outgroup is not shown here. Taxa names correspond to the molecular codes listed in Table 2.

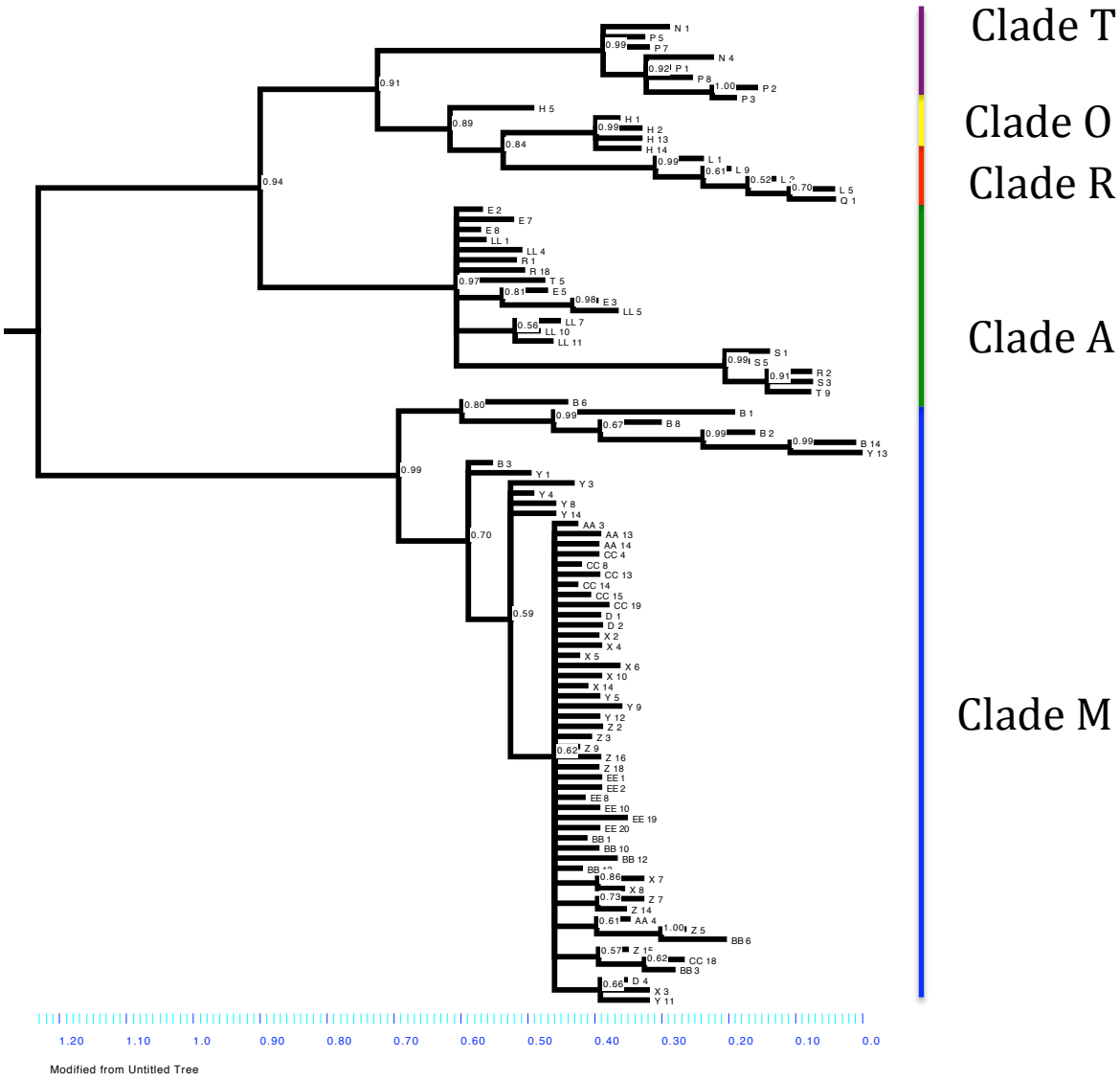

Figure 5
Mitochondrial D-loop phylogeny of *Notropis stramineus* populations produced by Bayesian Inference consensus of 37,500 trees. Nodal values represent posterior probabilities. The tree was rooted with *Cyprinella lutrensis*. The outgroup is not shown here. Taxa names correspond to the molecular codes listed in Table 2.

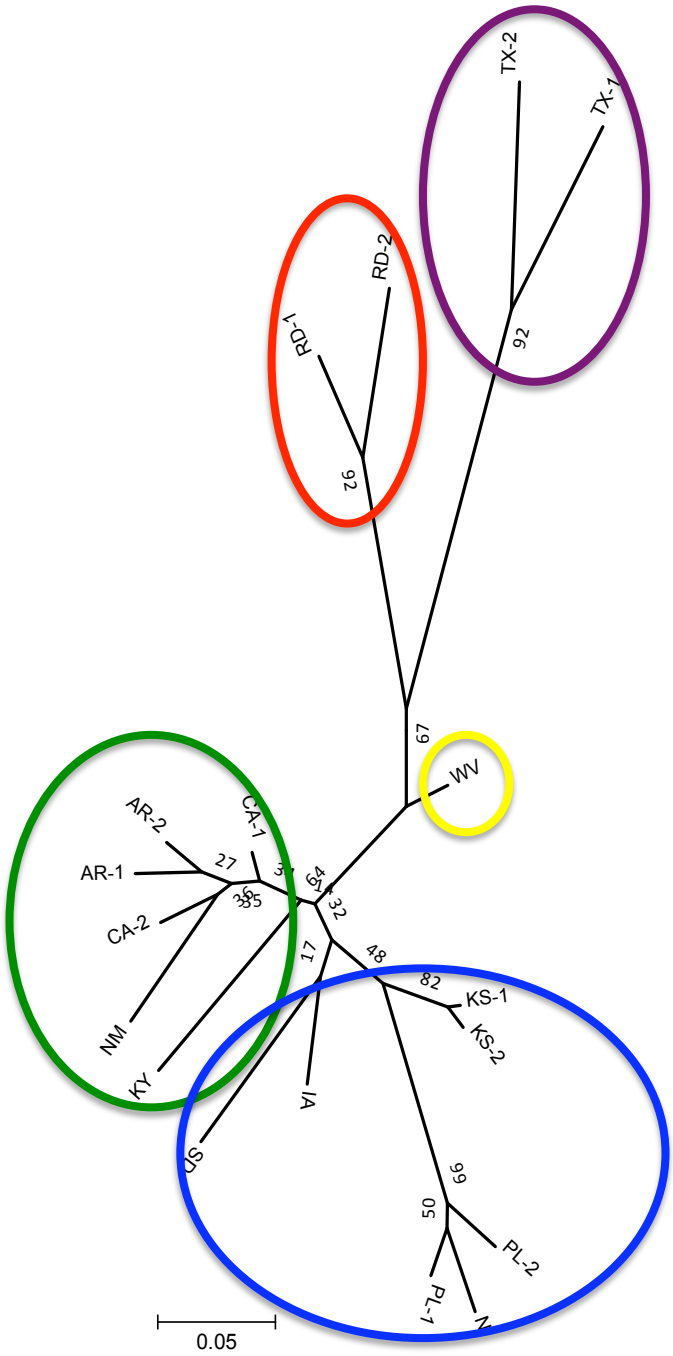

Figure 6
Neighbor joining tree constructed from microsatellite F_{ST} distances between populations of *Notropis stramineus*. Bootstrap values are indicated on branches. Abbreviations are the same as those in Table 2. Colors correspond to clades in Figures 4 and 5.

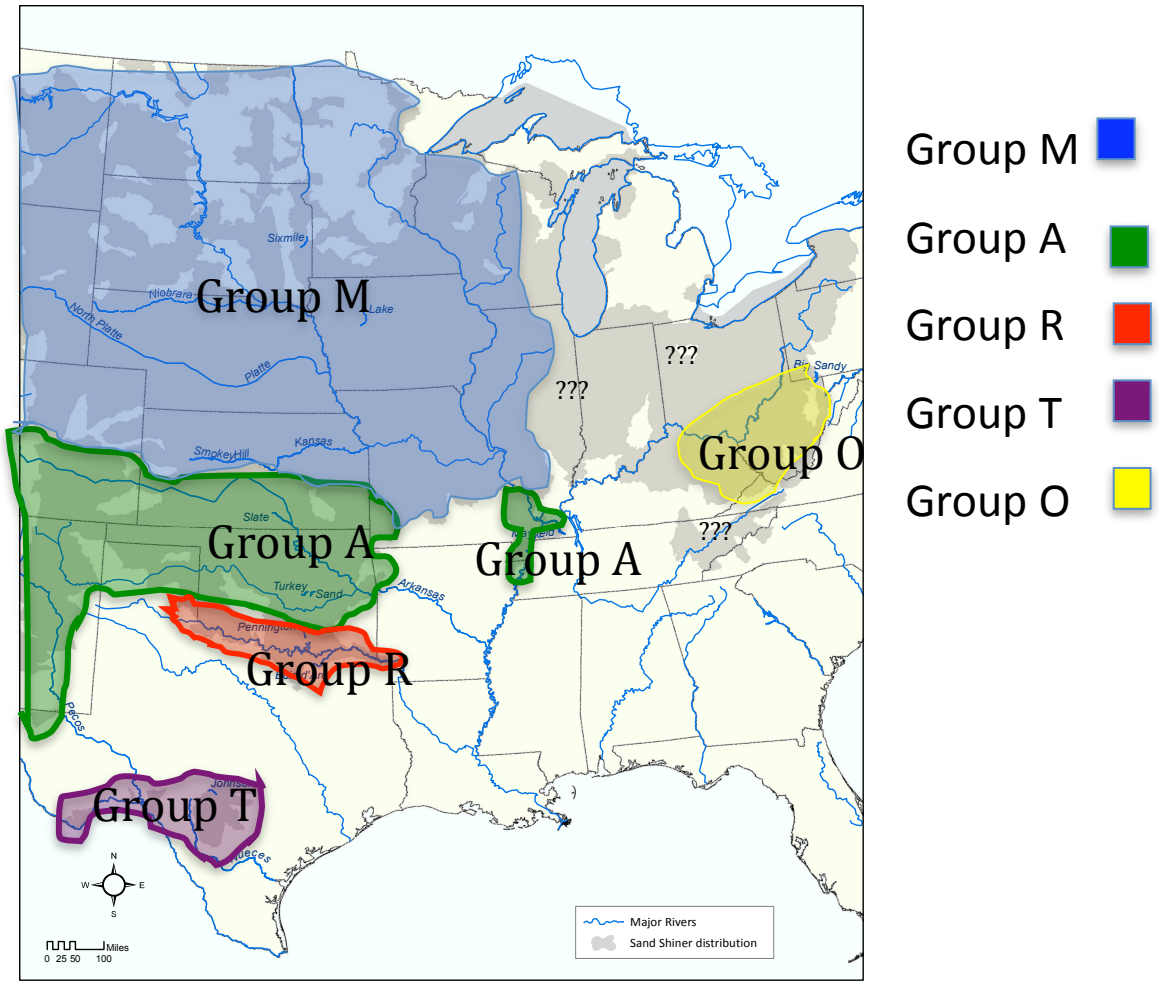

Figure 7
Current distribution of the five clades of the *Notropis stramineus* complex revealed by this study. Clade M may extend further east, and/or Clade O may extend further north, south, and west.

Introduction

The ability to identify and delineate species is central to all areas of biology because species are the fundamental unit of natural diversity (Harrison 1998). If we are to describe, understand, and conserve biodiversity, we must first be able to identify it. While most species have been identified and described on the basis of morphology, biological diversity is not limited to morphology. Investigations of behavior, natural history and genetics have all been used to uncover species-level diversity (Hillis 1988, Highton et al. 1989, Burbrink 2002, Wiens and Penkrot 2002, Johnson et al. 2004). Studies continue to uncover this "cryptic" diversity, especially with the advent of molecular methods.

Bickford et al. (2007) define the term "cryptic species" as two or more species that are or have been classified as a single nominal species because they are, at the least, indistinguishable on the basis of superficial morphology. Once identified as two or more unique species through non-morphological means (e.g. genetics or behavior), effort is rarely taken to discern whether morphological variation might actually exist between the cryptic species that may have been previously overlooked (Berendzen et al. 2009). Species can and have been diagnosed exclusively on the basis of genetics (Highton et al. 1989, Egge and Simons 2006) but it is a rare occurrence. While morphological divergence is not necessary to delineate species under many species concepts (e.g. evolutionary species concept, phylogenetic species concept, general lineage concept), diagnosable morphological characters make species descriptions accessible because morphological divergence has been the traditional means for describing species.

A previous morphological study of *N. stramineus* by Tanyolac (1973) produced a diagnosis of two subspecies based on 5 characters. In chapter two, I tested the hypothesis

that these subspecies represent distinct evolutionary lineages using an analysis of molecular variance and was able to reject the validity of the subspecies. Additionally, I provided evidence that *N. stramineus* is actually a complex of several cryptic species. Five different clades occupy the following drainages: Upper Mississippi River (Clade M), Arkansas, Canadian, and Pecos Rivers (Clade A), Red River (Clade R), Texas Gulf Coast (Clade T), and Ohio River (Clade O). These newly discovered lineages may contain previously undetected morphological variation that can corroborate the molecular evidence for their designation as separate species. Additionally, morphological investigations of these lineages may reveal diagnosable morphological characters that can be applied in field identification. While allopatric cryptic species can be identified on the basis of geographical location, it remains to be seen if all species of the *N. stramineus* complex exist in complete allopatry.

In this study, I investigate whether or not the newly discovered cryptic Great Plains clades of the *N. stramineus* species complex have experienced morphological divergence along with genetic divergence. Specifically, I test the hypothesis that these clades are not truly cryptic but are morphologically distinct. These clades may not have experienced any divergence in morphological characters. When diagnosable morphological characters are lacking between cryptic species, this finding is often explained by recent divergence time. This may be true for the majority of taxa but the discovery of anciently diverged cryptic species, most notably the bonefishes (Colborn et al. 2001), demonstrates that recent divergence time cannot automatically be assumed as an explanation for why diagnosable morphological characters have not yet evolved (Bickford et al. 2007). Morphological stasis between cryptic species can occur due to similar selection pressures. Additionally, non-

visual mating systems can lead to species divergence through signals (e.g. pheromones or vocalizations) without any changes in the morphological features that produce those signals (Pineiro et al. 1993, Gerhardt 2005).

Alternatively, the clades of the *N. stramineus* complex may have differences in shape that have been overlooked because of their subtlety and/or complexity. Variation in body shape is one of the most difficult types of variation to quantify (Parsons et al. 2003). Shape is a complex character as it consists of a multitude of linear distances that have to be evaluated as a whole. Differences in discrete, single traits (if they exist) are far easier to identify than differences in body shape. Unless shape differences are extreme, they usually cannot be detected with simple visual inspection.

In this study, I apply the methodology of traditional morphometrics to quantify body shape in the *N. stramineus* species complex. Specifically, I use Discriminant Function Analysis to evaluate whether or not a set of morphological variables indicative of shape can discriminate between the four clades of *N. stramineus* found in the Great Plains physiogeographic province. This statistical approach has been successfully used to distinguish between different populations and species in a variety of fishes (e.g. Berendzen et al. 2008, Reyes-Valdez et al. 2011).

Materials and Methods

I measured 29 linear body distances on 93 preserved individuals of *N. stramineus* from 9 distinct populations representing the four Great Plains clades identified in Chapter 2. Each population contained 9-12 individuals. A list of specimens and complete locality information is presented in Appendix 1 (Material Examined). Three populations were sampled from both Clades M and Clade A, two populations were sampled from Clade T, and one population was sampled from Clade R. The reason for this inequality in population sample size is because my preliminary phylogenetic analysis of mtDNA grouped Clades R and T into a single clade. Thus, there are no replicate populations in this study for Clade R. While more populations would allow for a greater generalization, it does not invalidate the following statistical analysis as unequal samples size among natural groups is allowed in Discriminant Function Analysis.

Morphological measurements were based on the box truss protocol of Bookstein et al. (1985) and the standardized method of Hubbs and Lagler (1958). Nineteen measurements were based on distances between 10 presumptive homologous landmarks and are described in Figure 2. Additional measurements include standard length (SL), head width (taken at occiput), pectoral-fin length, pelvic-fin length, dorsal-fin length (depressed), anal-fin length (depressed), snout length, bony width orbit, snout depth (taken vertically at nares), and transverse head depth (taken vertically at eyes). All measurements were taken on the left side of each fish using digital calipers with an accuracy of 0.01mm.

All specimens were adults with a SL larger than 30 mm. An assumption of no sexual dimorphism was made because *N. stramineus* has never been shown to have sexual dimorphism in any shape character. The only sexual dimorphic trait to be identified in *N.*

stramineus is the display very small tubercles on the heads and pelvic-fins of breeding males (Cross and Collins 1995).

In order to separate shape from size variation, all variables were divided by standard length to produce a body proportion ratio. All landmark-based morphometric methods face the challenge of removing variation due to size. No method is completely satisfactory in removing the effect of size without removing information about shape, therefore I used the simplest method.

A Discriminant Function Analysis (DFA) was performed with SAS/STAT® using 28 body proportions. DFA is a statistical method used to identify which continuous variables discriminate between two or more natural groups. The natural groups in this study are the clades recovered from the phylogenetic analyses in Chapter 2. Computationally, a DFA can be viewed as a MANOVA in reverse. DFA involves 3 major steps. First, a set of independent continuous variables is selected. The selected variables impart a statistically significant effect on the dependent categorical variables. The statistical significance is indicated by its *F* value. Second, canonical discriminant functions, also referred to as canonical variables (CVs) or roots, are extracted from the selected variables. The number of functions is equal to the number of groups minus one, or the number of variables in the analysis, whichever is smaller. Finally, the CVs are used to perform a discriminant analysis to classify individuals into groups. The percentage of correctly classified individuals reflects the strength of the CVs to discriminate between groups (Hill and Lewicki 2007).

Results

Raw measurements and ratios with SL of all individuals are presented in Appendix 5. Forward, backward, and stepwise methods of variable selection all returned the same set of 14 variables. The forward stepwise selection summary is presented (Table 2). It should be noted that statistical significance values of the selection process cannot be judged at face value because of the component of chance involved in the stepwise "pick and choose" method. The significance levels do not reflect the true alpha error rate (Hill and Lewicki 2007). The two variables showing the most overall effect in discriminating between groups are "occiput to pectoral origin/SL" (F= 30.06) followed by "pectoral-fin length/SL" (F=17.52).

Three CVs were computed using the 14 selected variables. The standardized canonical coefficients for each of the three CVs are shown in Table 2. Discriminant analysis using the three CVs successfully categorized 100% of individuals into their given natural group (e.g. clade) (Table 3).

The importance of each variable in each CV can be evaluated by looking at the size of the canonical coefficients. CV1 contributed 56.64% to the variation between clades and is predominately discriminatory with the following variables: occiput to pectoral origin, ventral peduncle length, and head width. CV 2 contributes another 23.66% to variation and is predominantly discriminatory with snout depth, prepelvic length, and bony width orbit. CV3 is responsible for the remaining 19.7% of variation and is predominantly discriminatory with pectoral-fin length and anal-fin length.

Class means on CVs for each clade are presented in Table 4. CV 1 is responsible for separating Clade A from Clade M, Clade A from a group composed of Clades R and T, and Clade M from a group composed of Clades R and T. CVs 2 and 3 are responsible for

separating Clade R from Clade T, Clade R from a group composed of Clades M and A, and Clade T from a group composed of Clades M and A. The differences between clades are easiest to visualize by plotting the CVs (from individual specimens) against each other. The plot of CV1 vs. CV 2 (Figure 3) clearly shows each clade forming a distinct cluster. The plot of CV 2 vs. CV 3 (Figure 4) is able to distinguish between 3 clusters - Clade R, Clade T, and group composed of Clades A and M. The plot of CV 1 vs. CV 3 (Figure 5) is able to distinguish between 3 clusters – Clade M, Clade A, and a group composed of Clades R and T. Though Clade A does have a small amount of overlap with Clades R and T, Clade A is clearly distinguishable as a group.

Discussion

Subtle, discernable shape differences exist between the four Great Plains clades in the *N. stramineus* complex and provide additional support for their recognition as distinct species. The statistical methods used by Tanyolac (1973) in the designation of *N. stramineus* subspecies were not powerful as they were greatly limited by the computing power of the 1970's, thus Tanyolac's interpretation involved a lot of speculation. Additionally, the study was not very extensive; it only incorporated a small set of morphological characters. The study presented here is far more extensive and uses appropriate statistical techniques. Discriminant Function Analysis was able to successfully discriminate between all four clades on the basis of 14 continuous morphological characters. While three CVs were found, the first two CVs alone are enough to distinguish between all four clades (Figure 3). Together, these two CVs contain 80.30% of the variation between clades. Additionally, CV 2 suggests similarities between Clades A and M while CVs 1 and 3 suggest similarities between Clades R and T (Table 3). This implies a geographical component to morphological variation between clades that warrants further inquiry.

This study did not consider morphological variation in populations of *N. stramineus* east of the Great Plains but I expect that populations from the Ohio River drainage (CLADE O) will also be distinguishable from other clades based on shape. Addition of these populations may also provide some resolution as to whether or not Clade R and Clade O share a common ancestry as discussed in Chapter 2.

Shape differences between clades of the *N. stramineus* species complex are reflective of divergences in fin placement (occiput to pectoral origin and prepelvic length), fin length (pectoral, pelvic, and anal), and skull structure (bony width orbit, width of head and depth

of snout). These changes are not drastic but can provide a starting point for identifying diagnostic morphological characteristics for species descriptions. I propose that comparisons of fin characteristic between clades will prove to be quite useful in this endeavor. Studies of behavior, reproduction and ecology could also be beneficial in further delineating these species. Little is known about the natural history of the different clades because all natural history studies of *N. stramineus* but one (Platania and Altenbach 1998) have been on Clade M populations (Summerfelt and Minckley 1969, Tanyolac 1973, Mueller and Pyron 2009, Smith et al. 2010).

The capability of the Discriminant Function Analysis to correctly categorize 100% of individuals into their respective clades suggests that this suite of variables will have great use in future studies to delineate species boundaries where the distributions of Clade A and Clade M meet along the Mississippi River. In chapter two, I presented evidence of possible asymmetric mitochondrial introgression from Clade M into Clade A in a population along the Mississippi River. Introgression implies past hybridization between the clades. Further study is necessary to quantify the amount and direction of hybridization and to establish if hybridization between these clades is an ongoing process. Morphological shape analyses can be complimentary to genetic investigations by providing an additional tool to ascertain whether or not a current hybrid zone exists between species. The ability to morphologically distinguish between Clade M and Clade A will be especially important if the degree or direction of hybridization is questionable. Crespin et al. (1999) successfully used a Discriminant Function Analysis to identify hybrids in their study of introgression in a cyprinid hybrid zone. When considering gene flow, hybrids can be of various types and form a continuum between two parental genetic pools. If a correlation exists between

genetic and morphological hybridization indices, morphology can be used to access the degree of hybridization of a given individual (Crespin et al. 1999).

In conclusion, this study provides strong evidence that the clades of the *N. stramineus* species complex in the Great Plains have accumulated changes in their morphology that parallel genetic divergence. Additional investigations will be needed to determine if the morphological changes are adaptive, the result of phenotypic plasticity due to subtle differences in the watersheds inhabited by these species, or merely the consequence of morphological drift. Fin characteristics, in particular, may be subject to adaptation and/or selection pressures. In fishes, fins are used for movement and stability, and the placement of fins on the body is partially reflective of the flow regime that a species inhabits. If there are differences in flow regime between the drainages inhabited by each clade, either selection or phenotypic plasticity could be responsible for the differences in fin morphology seen between the clades. Artificial selection experiments and the rearing of populations under different environmental conditions could reveal if selection, phenotypic plasticity, or both are acting to induce differences in fin characteristics between clades. Alternatively, the differences between clades may simply reflect genetic drift in genes related morphological traits. Felley (1984), Wood and Bain (1995), and Leavy and Booner (2009) did not detect any relationships between morphology and habitat selection within the stream-dwelling cyprinid species they studied. This suggests that differences in morphology between the clades of *N. stramineus* may be independent of flow regime and are, instead, the effect of drift.

The current study also confirms the utility of Discriminant Function Analysis in testing molecular hypotheses of cryptic divergence as put forth by Berendzen et al. (2009).

In their study, Berendzen et al. (2009) found discernable shape differences between cryptic clades of the *N. rubellus* species complex using Discriminant Function Analysis (though not as strong as in this study). Discriminant Function Analysis is not restricted to morphological analyses. Ramasindrazana et al. (2011) were able to discriminate between cryptic lineages of bats using bioacoustic parameters of echolocation calls. Because biological diversity is not restricted to morphology and molecules, future studies seeking to delineate cryptic species could benefit by applying Discriminant Function Analysis to an array of non-morphological characters. Finally, there is one major caveat to the results of this study. Although previous studies of *N. stramineus* have not revealed any sexual dimorphism other than breeding tubercles, it may have been overlooked, just as shape variation among the clades had been overlooked. It is possible, though not probable, that sex ratio bias (due to the relatively small within population sample sizes used in this study) could account for the patterns of morphological divergence between the clades of *N. stramineus.* Future analyses of the morphology of *N. stramineus* should include sex determination and explicit tests of sexual dimorphism. Discriminant Function Analysis of each clade with individuals grouped according to sex may reveal subtle sexual dimorphism in shape.

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Table 1
Summary statistics calculated for the 14 selected variables at each step in the forward stepwise selection process of the discriminant function analysis of *Notropis stramineus* morphology. Numbers in parenthesis refer to landmark measurements in Figure 2.

Step	Entered	Partial R-Square	F Value	P	Wilks' Lambda	P	Average Squared Canonical Correlation	P
1	(1-8)/SL	0.5033	30.06	<0.0001	0.49669338	<0.0001	0.16776887	<0.0001
3	(5-4)/SL	0.2710	10.78	<0.0001	0.22670718	<0.0001	0.31998153	<0.0001
4	(10-1)/SL	0.2686	10.53	<0.0001	0.16580777	<0.0001	0.39023994	<0.0001
5	Snout Depth/SL	0.2173	7.87	0.0001	0.12977793	<0.0001	0.45304399	<0.0001
6	Bony width orbit/SL	0.2355	8.62	<0.0001	0.09922081	<0.0001	0.51220781	<0.0001
7	Anal-fin Length /SL	0.2429	8.87	<0.0001	0.07512295	<0.0001	0.56355531	<0.0001
8	Head Width/SL	0.1868	6.28	0.0007	0.06109111	<0.0001	0.58343816	<0.0001
9	(7-9)/SL	0.1848	6.12	0.0008	0.04980395	<0.0001	0.61299108	<0.0001
10	(8-7)/SL	0.2281	7.88	0.0001	0.03844170	<0.0001	0.64987663	<0.0001
11	(2-3)/SL	0.1533	4.77	0.0042	0.03254870	<0.0001	0.66424575	<0.0001
12	Snout Length/SL	0.1444	4.39	0.0066	0.02784732	<0.0001	0.67820378	<0.0001
13	(1-2)/SL	0.0792	2.21	0.0941	0.02564296	<0.0001	0.68875938	<0.0001
14	(10-9)/SL	0.0810	2.23	0.0911	0.02356539	<0.0001	0.69793789	<0.0001

Table 2
Total-sample standardized canonical coefficients for the 14 selected morphological variables on each of three canonical variables (Can1, Can2, and Can3) from the discriminant function analysis of *Notropis stramineus*. Numbers in parentheses after a variable refer to landmarks in Figure 2. SL= standard length.

Variable	Can1	Can2	Can3
Occiput to Pectoral Origin (1-8)/SL	1.013209696	0.022086272	-0.194331586
Pectoral-fin Length/SL	0.488427116	0.283214914	0.804757240
Ventral Peduncle Length (5-4)/SL	1.103528582	0.123571747	-0.080225948
Dorsal Head Length (10-1)/SL	-0.067357658	-0.511702329	-0.622225080
Snout Depth/SL	-0.130023661	1.782534251	-1.068212064
Bony width orbit/SL	-0.479385730	0.949280622	-0.457759259
Anal-fin Length (depressed)/SL	-0.248973635	-0.531894254	0.756788865
Head Width/SL	0.811408596	-0.362710526	0.119797929
Prepelvic Length (7-9)/SL	-0.133941901	0.978997832	0.206320050
Pectoral to Pelvic Origin (8-7)/SL	-0.052302930	-0.780291731	0.084572138
Dorsal Fin Base (2-3)/SL	0.509743760	0.267444499	0.100134621
Snout Length/SL	-0.409972850	-0.190605231	0.271030797
Predorsal Length (1-2/)/SL	0.095305822	0.234172219	0.391916614
Ventral Head Length (10-9)/SL	0.248122961	-0.211832924	0.362491782
Eigenvalue	4.5953	1.9188	1.5984
Cumulative Proportion	0.5664	0.803	1.00

Table 3
Classification success of individuals into predicted clades based on discriminant functions. All individuals were correctly classified into their respective clades.

Number of Observations and Percent Classified into Groups					
From Group	A	M	R	T	Total
Clade A	33 100.00	0 0.00	0 0.00	0 0.00	33 100.00
Clade M	0 0.00	29 100.00	0 0.00	0 0.00	29 100.00
Cade R	0 0.00	0 0.00	10 100.00	0 0.00	10 100.00
Clade T	0 0.00	0 0.00	0 0.00	21 100.00	21 100.00
Total	33 35.48	29 31.18	10 10.75	21 22.58	93 100.00

Table 4
Group means for each clade of *Notropis stramineus* on each canonical variable from the discriminant function analysis of 14 selected morphological variables.

Clade	CV 1	CV 2	CV 3
A	2.329521625	0.311420309	0.901549975
M	-2.811275920	0.249852780	0.758109120
R	0.130482786	2.684066878	-2.586268064
T	0.159426675	-2.112536649	-1.232077762

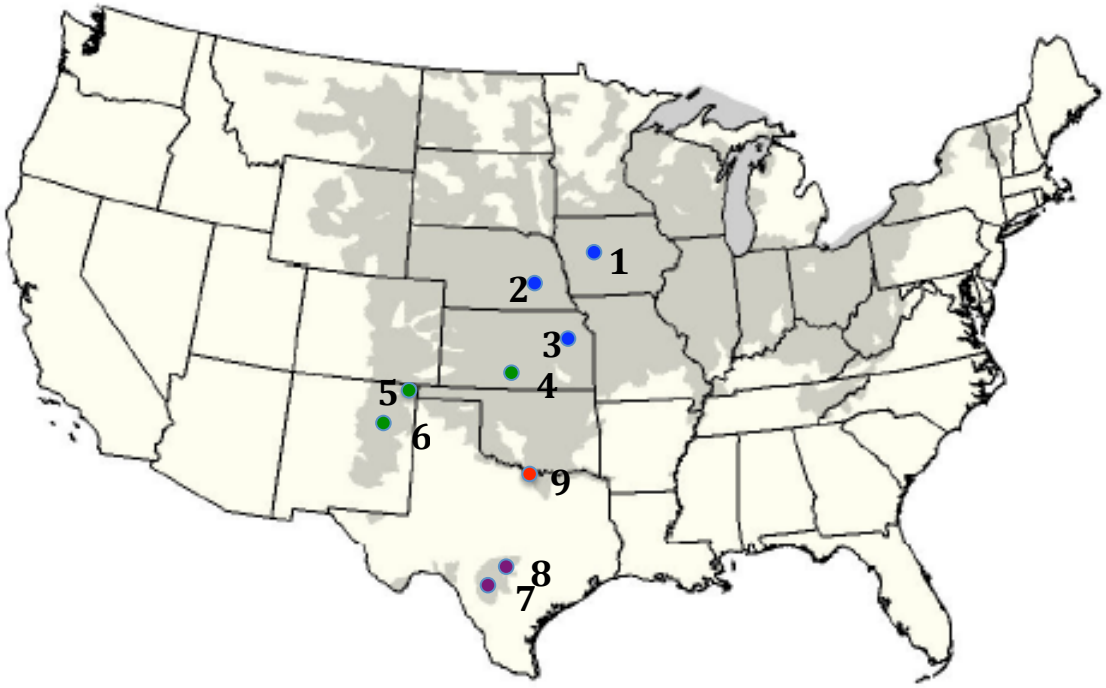

Figure 1
Distribution of *Notropis stramineus* (shaded area) and populations sampled for morphological analysis. 1 – Lake Creek, IA; 2 – Platte River, NE; 3 – Kansas River, KS; 4 – Arkansas River, KS; 5 – Conchas River, NM; 6 – Pecos River, NM; 7 – Frio River, TX; 8 – Sandy Creek, TX; 9 – Clear Boggy Creek, OK. Populations 1-3 are from Clade M, populations 4-6 are from Clade A, populations 7-8 are from Clade T, and population 9 is from Clade R.

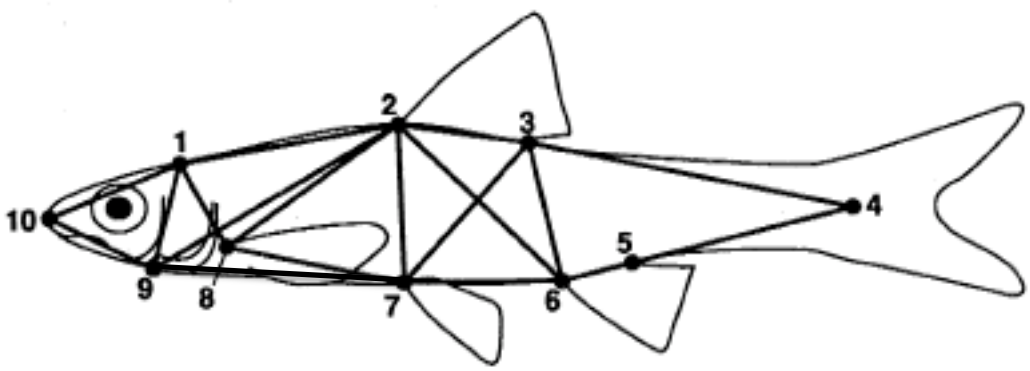

Figure 2
Homologous landmarks used for box truss morphometric measurements (modified from Warren et al. 1994). Descriptions of distances between landmarks are as follows: **10-1** = dorsal head length (tip snout to occiput), **10-9** = ventral head length (tip snout to isthmus), **1-9** – head depth (occiput to isthmus), **1-8** = occiput to pectoral origin, **1-7** = occiput to pelvic origin, **1-2** = predorsal length (occiput to dorsal-fin origin), 2**-9** – dorsal origin to isthmus, **2-8** = dorsal to pectoral origin, **2-7** = predorsal depth, **2-8** = dorsal to anal origin, **2-3** = dorsal-fin base, 3**-7** = posterior dorsal to pectoral origin, **3-6** = postdorsal depth (posterior dorsal-fin base to anal-fin origin), **3-4** = dorsal peduncle length, **5-4** = ventral peduncle length, **6-5** = anal fin base, **7-6** = postpelvic length, **8-7** = pectoral to pelvic origin, **7-9** = prepelvic length (pelvic-fin origin to isthmus).

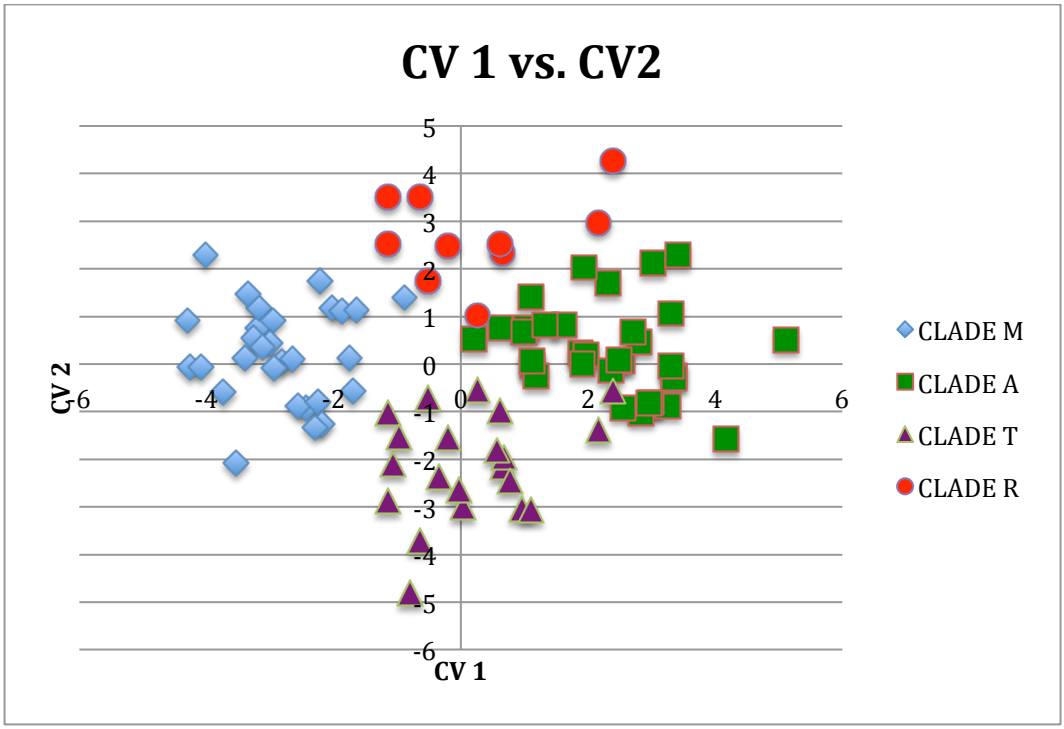

Figure 3
Plot of the first and second canonical variables from the discriminant function analysis of 14 morphological variables in four clades of *Notropis stramineus*.

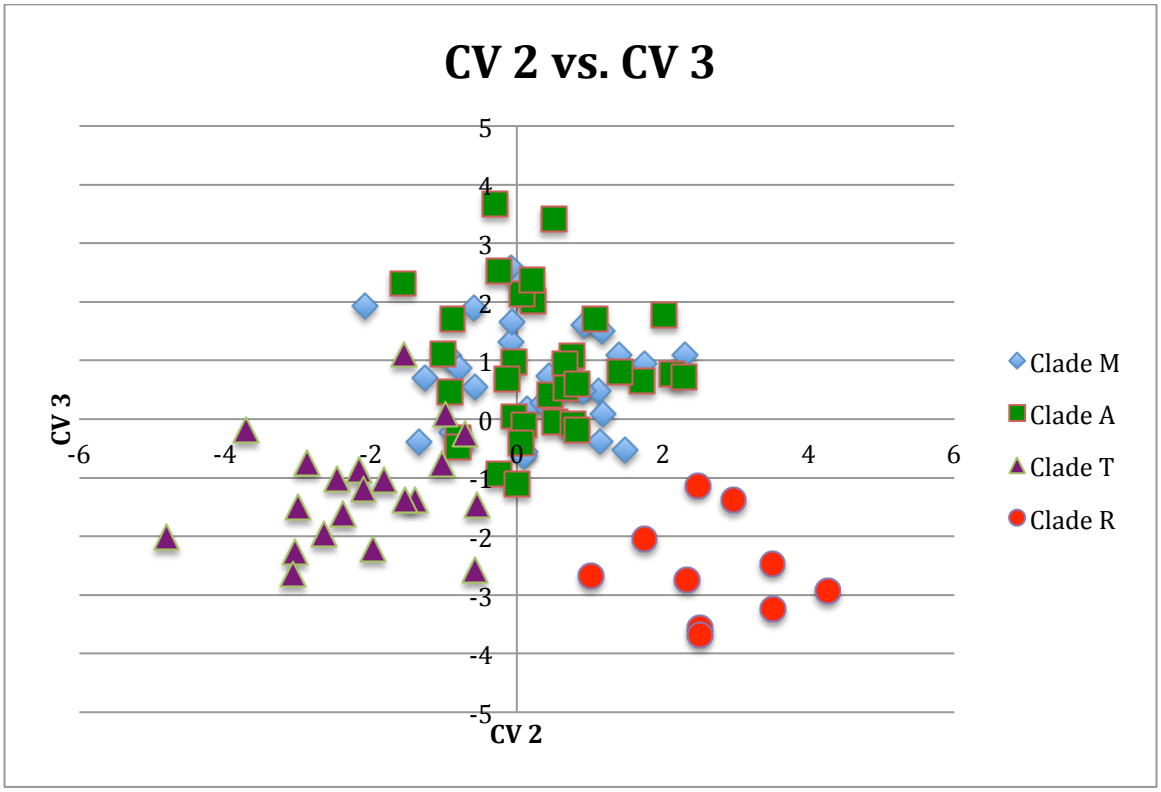

Figure 4
Plot of the second and third canonical variables from the discriminant function analysis of 14 morphological variables in four clades of *Notropis stramineus*.

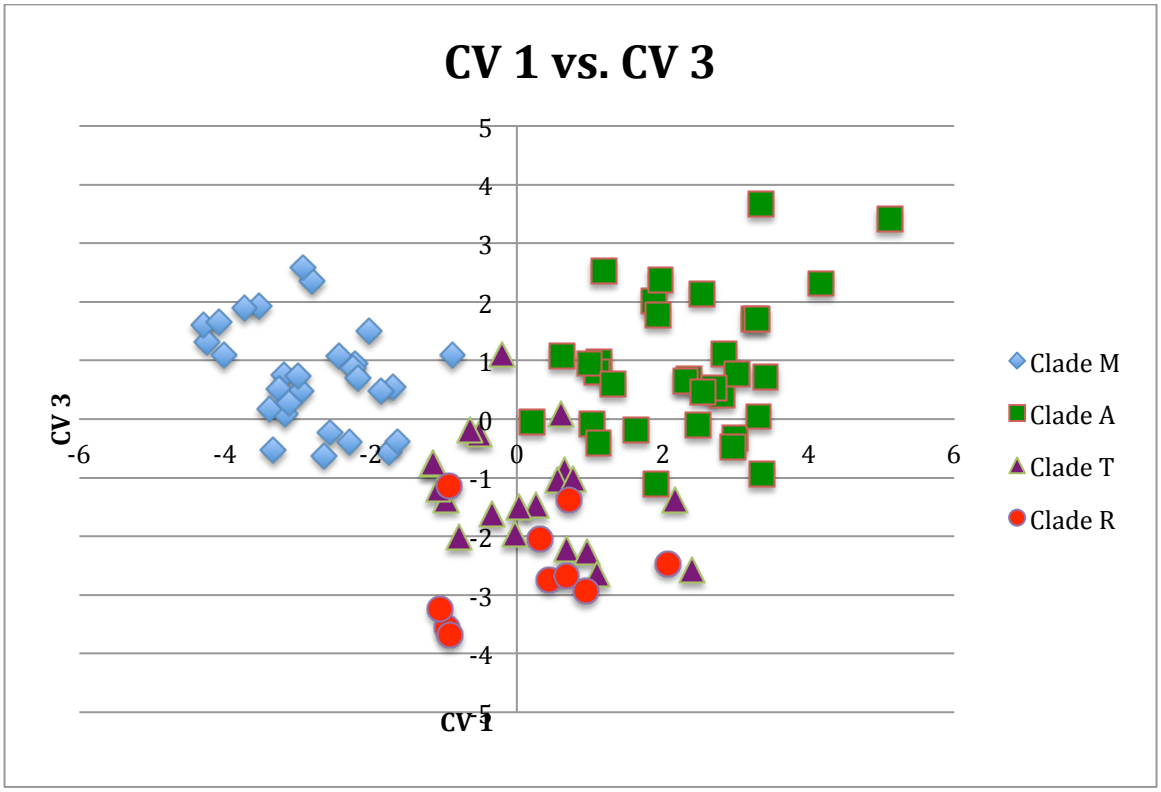

Figure 5
Plot of the first and third canonical variables from the discriminant function analysis of 14 morphological variables in four clades of *Notropis stramineus*.

Appendix 1
Materials examined
Fish examined in this dissertation included both field collected and museum specimens. The specific locations of collection and the number of individuals (N) for each location are listed for each chapter along with the year of collection.

Chapter 1

Sample site	N	River	County	State	Voucher or Field Number	Latitude ° N	Longitude ° W	Year
Russell	10	Smoky Hill River	Russell	KS	KU 29230	38.882999	98.849999	1998
Enterprise	20	Smoky Hill River	Dickinson	KS	KJP09-1	38.902276	97.167871	2009
Manhattan	20	Kansas River	Riley	KS	KJP09-2	39.173433	96.554332	2009
Topeka	20	Kansas River	Shawnee	KS	KJP09-3	39.074194	95.716389	2009
Mill Creek	3	Kansas River	Wabaunsee	KS	KU 29218	39.003899	96.281097	1998
Mill Creek	3	Kansas River	Wabaunsee	KS	KU 29219	39.003899	96.281097	1998
Mill Creek	11	Kansas River	Wabaunsee	KS	KU 29220	39.003899	96.281097	1998
Lawrence	5	Kansas River	Douglas	KS	KJP02-6	38.977083	95.234528	2002
Lawrence	14	Kansas River	Douglas	KS	KJP07-1	38.977083	95.234528	2007

KU – University of Kansas Natural History Museum, KJP – Kendra J. Pittman

Chapter 2

Clade	N	River	County	State	Voucher or Field Number	Latitude ° N	Longitude ° W	Year
A	20	Mayfield Creek	Graves	KY	KJP02-3	36.855667	88.634250	2003
M	11	Lake Creek	Calhoun	IA	KR-06	42.549999	94.667000	2006
M	13	Six Mile Creek	Brookings	SD	CRB-06	44.549992	96.633003	2006
M	8	Niobrara River	Cherry	NE	KJP07-2	42.852389	100.758778	2007
M	10	Platte River	Sarpy	NE	KJP07-4	41.015222	96.158000	2007
M	20	North Platte River	Lincoln	NE	KJP07-3	41.153889	100.758778	2007
M	5	Kansas River	Douglas	KS	KJP02-6	38.977083	95.234528	2002
M	14	Kansas River	Douglas	KS	KJP07-1	38.977083	95.234528	2007
M	17	Smoky Hill River	Dickinson	KS	KJP09-1	38.902276	97.167871	2009
A	7	Slate Creek	Cowley	KS	KJP02-7	37.158583	97.177389	2007
A	12	Arkansas River	Rice	KS	KU 29229	38.233002	98.300031	1998
A	11	Sand Creek	Seminole	OK	PU05-13	35.347953	96.494358	2005
A	14	Turkey Creek	Seminole	OK	PU05-14	35.348484	96.691436	2005
A	18	Pecos River	De Baca	NM	MSB 54589	34.465613	104.245920	2006
R	10	Pennington Creek	Johnston	OK	PU04-30	34.220409	96.700544	2004
R	9	Bois D'Arc Creek	Fannin	OK	PU04-60	33.550000	96.183333	2004
O	19	Big Sandy Creek	Preston	WV	KJP05-5	39.687444	79.627028	2005
T	9	Johnson Creek	Kerr	TX	PU04-40	30.141667	99.333333	2004
T	4	Nueces River	Uvalde	TX	PU04-37	29.616667	100.008333	2004

KU – University of Kansas Natural History Museum, MSB – Museum of Southwest Biology, KJP – Kendra J. Pittman, PU – Peter Unmack, KR – Kevin Roe, CRB – Charles R. Berry

Chapter 3

Clade	N	River	County	State	Voucher or Field Number	Latitude ° N	Longitude ° W	Year
M	10	Lake Creek	Calhoun	IA	KU 29244	42.549999	94.667000	1998
M	10	Platte River	Sarpy	NE	KJP07-4	41.015222	96.158000	2007
M	9	Kansas River	Douglas	KS	KU 29216	38.977083	95.234528	1998
A	13	Arkansas River	Rice	KS	KU 29229	38.233002	98.300031	1998
A	10	Conchas River	San Miguel	NM	MSB 78308	35.386911	104.460628	1988
A	10	Pecos River	Guadalupe	NM	MSB 79104	34.825250	104.625270	2010
T	10	Frio River	Uvalde	TX	TNHC 30249	29.495290	99.711628	2003
T	10	Sandy Creek	Llano	TX	KJP05-1	30.546611	98.543917	2005
R	6	Clear Boggy Creek	Pontotoc	OK	MSB 76731	34.659886	96.632545	1999
R	8	Clear Boggy Creek	Pontotoc	OK	MSB 76740	34.703328	96.634862	1999

KU – University of Kansas Natural History Museum, MSB – Museum of Southwest Biology, TNHC – Texas Natural History Collections, KJP – Kendra J. Pittman

Appendix 2
Microsatellite genotypes for samples of *N. stramineus* from the Kansas River. Alleles correspond to the size of each fragment (in bp).

Sample	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Russell	MM-1	231	246	111	215	229	218	174
		233	264	115	217	231	222	178
	MM-2	231	266	107	215	225	222	174
		231	274	109	215	229	222	174
	MM-3	231	258	103	215	221	220	174
		231	272	107	219	229	220	174
	MM-4	231	258	111	213	229	220	174
		231	270	115	213	235	220	174
	MM-5	227	?	109	213	221	218	174
		227	?	111	215	231	222	174
	MM-6	227	262	107	209	223	222	174
		231	272	107	211	237	222	174
	MM-7	227	258	111	223	225	222	174
		231	270	111	223	237	222	180
	MM-8	331	272	103	215	225	220	174
		231	280	109	219	231	222	174
	MM-9	227	250	111	215	223	222	174
		231	280	119	229	229	222	174
	MM-10	231	252	115	215	229	222	174
		231	256	123	229	235	222	174

? - indicates missing data

Sample	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Enterprise	EE-1	227	268	107	213	229	222	174
		233	270	109	215	233	222	174
	EE-2	227	266	105	207	223	222	174
		231	268	105	207	229	222	174
	EE-3	227	258	111	219	229	222	174
		231	260	111	223	229	222	174
	EE-4	229	264	107	213	229	222	174
		231	264	109	221	229	222	174
	EE-6	231	258	109	215	225	220	174
		231	264	123	217	229	222	176
	EE-7	231	258	109	217	221	220	174
		231	274	109	217	225	222	174
	EE-8	231	254	103	215	221	220	174
		231	264	103	221	225	222	174
	EE-9	227	270	107	211	223	222	174
		227	272	107	211	223	222	180
	EE-10	227	266	103	215	229	220	174
		231	266	103	227	229	220	174
	EE-11	231	264	115	229	231	222	174
		231	284	115	229	231	222	174
	EE-12	227	252	105	221	229	218	174
		231	292	113	221	233	222	174
	EE-13	227	266	109	213	233	222	174
		231	272	109	229	233	222	178
	EE-14	231	260	107	215	235	218	174
		231	284	119	237	235	222	174
	EE-15	231	256	97	229	229	222	174
		233	272	113	229	229	222	174
	EE-16	231	266	107	217	233	220	174
		231	276	107	221	233	222	174
	EE-17	231	260	103	213	233	222	174
		231	260	107	213	233	222	174
	EE-18	233	258	109	217	225	220	174
		239	272	111	217	229	222	174
	EE-19	231	256	103	225	229	220	174
		231	258	103	225	231	228	174
	EE-20	231	260	107	229	223	218	174
		231	262	109	229	229	220	174

Sample	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Manhattan	HH-1	227	254	107	229	223	220	174
		231	256	111	229	233	220	174
	HH-2	227	258	107	213	237	222	174
		231	264	107	225	239	222	178
	HH-3	227	266	107	215	225	222	174
		231	276	115	215	229	222	180
	HH-4	?	260	?	?	221	218	178
		?	260	?	?	225	220	180
	HH-5	231	252	107	215	223	222	174
		231	260	121	225	233	222	174
	HH-6	231	246	103	213	225	220	174
		231	246	107	213	225	222	174
	HH-7	?	254	?	?	221	220	174
		?	254	?	?	221	220	174
	HH-8	231	250	127	215	231	222	174
		231	250	127	215	231	222	174
	HH-9	231	254	111	213	229	218	174
		231	284	121	223	233	222	174
	HH-10	227	250	101	219	233	220	174
		227	254	107	219	233	220	174
	HH-11	227	250	113	215	229	220	174
		231	250	121	215	233	222	174
	HH-12	227	272	123	?	231	222	174
		231	280	123	?	233	228	174
	HH-13	231	258	101	219	229	222	174
		231	270	107	223	229	222	174
	HH-14	?	266	109	?	229	222	174
		?	266	109	?	233	222	174
	HH-15	227	260	113	219	229	220	174
		227	290	113	233	229	222	174
	HH-16	231	254	103	229	227	220	174
		231	270	111	229	227	220	176
	HH-17	227	258	103	223	231	222	174
		231	296	109	229	231	222	174
	HH-18	231	266	107	213	225	222	174
		231	270	123	225	229	222	174
	HH-19	227	256	107	219	225	222	174
		227	260	107	221	229	228	174
	HH-20	227	252	107	223	229	220	174
		231	258	107	223	233	220	174

? - indicates missing data

Sample	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Topeka	JJ-1	227	252	103	213	223	222	174
		231	278	117	219	225	222	174
	JJ-2	227	256	115	223	227	222	174
		227	264	115	225	231	222	178
	JJ-3	231	254	115	213	229	220	174
		231	288	115	217	237	222	174
	JJ-4	227	252	115	217	223	222	174
		231	264	127	217	229	222	174
	JJ-5	227	264	105	213	229	222	174
		231	264	117	225	229	222	178
	JJ-6	227	250	109	221	225	222	174
		227	256	123	225	229	222	174
	JJ-7	227	278	107	213	221	222	174
		233	278	109	229	229	222	174
	JJ-8	231	272	107	213	225	222	174
		231	280	107	215	233	222	180
	JJ-9	227	274	107	229	231	222	174
		231	292	111	229	243	222	174
	JJ-10	227	268	101	215	227	222	174
		231	270	107	225	233	222	174
	JJ-11	227	282	107	215	223	222	174
		231	298	107	229	229	220	174
	JJ-12	227	258	113	215	223	222	174
		227	262	113	229	233	222	174
	JJ-13	231	294	103	215	223	222	174
		231	296	127	215	223	222	174
	JJ-14	227	260	107	207	225	222	174
		231	262	107	217	229	222	174
	JJ-15	227	262	107	211	223	218	174
		227	266	107	213	223	222	180
	JJ-16	227	260	107	215	229	220	178
		231	268	115	219	229	222	178
	JJ-17	231	264	107	221	231	220	174
		231	266	111	221	231	222	174
	JJ-18	227	250	103	213	221	222	174
		231	278	109	235	229	222	174
	JJ-19	227	254	111	217	219	220	174
		231	294	121	217	231	222	174
	JJ-20	227	254	107	213	225	222	174
		231	296	107	219	225	222	174

Sample	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Mill Creek	KK-1	227	252	?	207	225	220	174
		231	256	?	213	229	222	178
	KK-2	231	254	109	217	225	218	174
		231	256	111	229	237	222	174
	KK-3	231	258	109	213	229	222	174
		231	262	109	215	233	222	174
	KK-4	227	270	?	219	229	220	174
		231	272	?	219	237	222	178
	KK-5	227	254	?	?	225	222	176
		227	254	?	?	225	222	176
	KK-6	231	264	111	215	225	222	174
		231	284	111	237	225	222	174
	KK-7	227	268	107	223	223	220	174
		231	284	119	225	237	220	174
	KK-8	227	264	103	213	225	220	174
		231	282	107	213	225	222	178
	KK-9	231	260	107	229	225	220	174
		239	270	115	229	225	222	174
	KK-10	231	250	107	215	223	220	174
		231	258	107	227	229	222	174
	KK-11	227	260	111	219	229	222	174
		227	272	111	219	229	222	174
	KK-12	231	256	105	215	223	222	174
		231	260	105	235	229	222	174
	KK-13	227	250	107	213	225	222	174
		231	256	109	217	229	222	174
	KK-14	227	266	107	213	223	222	174
		231	274	115	213	233	222	174
	KK-15	227	260	107	217	231	220	174
		231	260	107	223	249	218	174
	KK-16	231	250	121	219	223	220	174
		231	290	127	223	253	222	174
	KK-17	231	262	103	219	229	218	174
		231	266	111	227	229	222	174

? - indicates missing data

Sample	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Lawrence	D-1	227	254	107	213	225	220	174
		227	256	107	217	233	220	174
	D-2	231	260	103	213	229	222	174
		231	272	105	219	229	222	174
	D-3	231	264	107	215	221	222	174
		231	282	109	215	221	222	174
	D-4	227	256	107	213	231	220	174
		231	260	113	215	231	222	174
	D-5	227	254	111	211	229	222	174
		231	264	111	229	233	222	174
	X-1	231	250	123	221	221	218	174
		235	266	125	221	229	220	174
	X-2	231	256	107	225	223	224	174
		231	260	117	231	229	224	174
	X-3	?	278	107	215	229	222	174
		?	278	125	233	237	222	174
	X-4	231	258	103	221	231	222	174
		233	266	109	221	231	222	174
	X-5	227	264	109	213	221	218	174
		227	266	115	217	229	220	178
	X-6	237	254	119	219	227	222	174
		237	262	125	223	237	222	174
	X-7	229	256	109	217	223	220	174
		231	270	121	217	223	224	174
	X-8	231	246	107	213	229	220	174
		231	260	107	215	231	222	174
	X-9	231	258	105	225	229	222	174
		233	260	105	225	233	222	174
	X-10	231	264	109	213	225	218	174
		231	270	111	213	225	218	174
	X-11	227	260	107	213	229	222	174
		231	262	117	215	229	222	174
	X-12	231	254	103	221	227	222	174
		231	286	105	221	235	222	174
	X-14	239	266	109	221	229	220	174
		239	290	109	221	229	222	174
	X-15	?	256	?	213	225	220	174
		?	274	?	213	229	222	174
	x-16	?	260	109	?	225	?	174
		?	262	111	?	229	?	174

? - indicates missing data

Appendix 3
Haplotype frequencies in each sampled population.

		Population								
Haplotype	Genbank Number	SD	KY	PL-1	KS-1	AR-1	WV	RD-1	TX-1	AR-2
1		6			3					
2		3								
3		1								
4		3			1					
5			5							
6			4							
7			2							
8			2							
9			4							
10			3							
11										
12										
13										
14										
15										
16										
17										
18					1					
19					1					
20					3					
21						1				1
22						2				1
23						2				2
24						1				
25						1				
26							13			
27							3			
28							1			
29							1			
30							1			
31								1		
32								7		
33								1		
34								1		
35									2	
36									2	
37										2
38										1
39										2
40										1
41										1
42										1
43										
44									1	
45									1	

		Population								
Haplotype	Genbank Number	SD	KY	PL-1	KS-1	AR-1	WV	RD-1	TX-1	AR-2
46									2	
47									1	
48									1	
49										
50										
51										
52										
53										
54										
55										
56										
57					1					
58					1					
59					1					
60					2					
61					1					
62					1					
63					1					
64					1					
65										
66										
67										
68										
69										
70										
71										
72										
73										
74										
75										
76				2						
77				1						
78				1						
79				1						
80				1						
81				1						
82				1						
83				1						
84				1						
85										
86										
87										
88										
89										
90										
91										
92										

		Population								
Haplotype	Genbank Number	SD	KY	PL-1	KS-1	AR-1	WV	RD-1	TX-1	AR-2
93										
94										
95										
96										
97										

		Popluation								
Haplotype	Genbank Number	TX-2	RD-2	NM	CA-1	CA-2	IA	PL-2	KS-2	NI
1								9	5	3
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										1
12									1	1
13										1
14										1
15										1
16								1		1
17										1
18										
19									1	
20									2	
21										
22										
23					2	4				
24										
25					1					
26										
27										
28										
29										
30										
31										
32			6							
33										
34										
35										
36										
37										
38										
39										
40										
41						1				
42										
43		3								
44										
45										

		Population								
Haplotype	Genbank Number	TX-2	RD-2	NM	CA-1	CA-2	IA	PL-2	KS-2	NI
46										
47										
48										
49			3							
50				14						
51				3						
52				1						
53					3					
54					1	1				
55					4	6				
56						1				
57						1				
58									1	
59										
60										
61							1		1	
62										
63										
64										
65										
66										
67							1			
68							1			
69							1			
70							1			
71							1			
72							1			
73							1			
74							1			
75							1			
76							1			
77										
78										
79										
80										
81										1
82										1
83										
84										
85										
86									1	
87									1	
88									1	
89									1	
90									1	
91									1	
92										1

		Population								
Haplotype	Genbank Number	TX-2	RD-2	NM	CA-1	CA-2	IA	PL-2	KS-2	NI
93										1
94										1
95										1
96										1
97										1

Appendix 4
Microsatellite genotypes for samples of *N. stramineus*. Alleles correspond to the size of each fragment (in bp).

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Mayfield	B-2	231	256	107	217	227	222	176
Creek, KY		231	264	107	217	235	222	180
	B-4	231	256	105	213	227	220	178
		231	258	105	235	227	222	180
	B-5	231	254	103	219	235	220	174
		231	258	103	235	243	222	178
	B-6	231	252	121	211	231	220	174
		231	256	121	217	231	222	180
	B-7	227	258	115	205	229	222	176
		227	264	121	217	231	222	178
	B-8	231	256	117	213	227	222	178
		231	258	121	235	227	222	180
	B-9	231	252	?	219	227	220	174
		231	258	?	235	227	222	178
	B-12	231	252	107	205	227	220	174
		231	256	117	235	227	222	180
	B-13	231	252	113	211	227	220	174
		231	256	113	217	227	222	180
	B-14	231	252	105	231	227	220	174
		231	256	105	235	229	220	180
	B-15	231	264	105	235	227	220	176
		231	264	105	235	227	222	176
	B-16	231	250	105	205	227	222	178
		231	256	105	219	235	222	180
	B-17	231	256	107	217	229	220	178
		231	258	113	217	231	222	180
	B-18	231	256	111	235	225	222	174
		231	272	111	235	227	222	180
	B-19	231	256	105	213	227	222	176
		231	264	109	219	227	222	180
	B-20	231	256	111	231	227	220	180
		231	260	121	231	231	220	180
	B-21	231	256	103	213	221	220	176
		231	264	121	235	227	222	180
	B-22	231	252	115	217	229	220	174
		231	254	121	217	243	220	174

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Kansas	D-1	227	254	107	213	225	220	174
River, KS		227	256	107	217	233	220	174
	D-2	231	260	103	213	229	222	174
		231	272	105	219	229	222	174
	D-3	231	264	107	215	221	222	174
		231	282	109	215	221	222	174
	D-4	227	256	107	213	231	220	174
		231	260	113	215	231	222	174
	D-5	227	254	111	211	229	222	174
		231	264	111	229	233	222	174
	X-1	231	250	123	221	221	218	174
		235	266	125	221	229	220	174
	X-2	231	256	107	225	223	224	174
		231	260	117	231	229	224	174
	X-3	?	278	107	215	229	222	174
		?	278	125	233	237	222	174
	X-4	231	258	103	221	231	222	174
		233	266	109	221	231	222	174
	X-5	227	264	109	213	221	218	174
		227	266	115	217	229	220	178
	X-6	237	254	119	219	227	222	174
		237	262	125	223	237	222	174
	X-7	229	256	109	217	223	220	174
		231	270	121	217	223	224	174
	X-8	231	246	107	213	229	220	174
		231	260	107	215	231	222	174
	X-9	231	258	105	225	229	222	174
		233	260	105	225	233	222	174
	X-10	231	264	109	213	225	218	174
		231	270	111	213	225	218	174
	X-11	227	260	107	213	229	222	174
		231	262	117	215	229	222	174
	X-12	231	254	103	221	227	222	174
		231	286	105	221	235	222	174
	X-14	239	266	109	221	229	220	174
		239	290	109	221	229	222	174
	X-15	?	256	?	213	225	220	174
		?	274	?	213	229	222	174
	x-16	?	260	109	?	225	?	174
		?	262	111	?	229	?	174

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Slate	E-1	229	260	107	213	229	220	178
Creek, KS		229	282	119	213	229	222	178
	E-2	231	258	109	215	229	218	174
		237	284	109	215	231	222	178
	E-3	231	256	113	215	229	218	178
		231	262	113	219	229	222	180
	E-4	231	256	107	?	229	218	174
		231	258	107	?	229	218	178
	E-5	229	266	113	213	225	218	174
		231	270	113	213	229	218	178
	E-6	229	258	105	?	225	220	180
		231	262	105	?	225	222	180
	E-7	225	258	109	217	229	220	174
		231	272	109	217	229	220	178
	E-8	231	262	103	213	233	218	174
		231	274	121	213	249	220	180

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Arkansas	LL-1	229	266	115	?	229	220	174
River, KS		231	270	117	?	235	220	180
	LL-2	229	264	105	?	225	222	174
		229	266	107	?	229	222	180
	LL-3	231	258	103	219	229	218	178
		231	264	107	219	229	224	180
	LL-4	229	258	109	217	225	222	178
		229	264	113	217	229	222	178
	LL-5	231	268	105	213	229	218	174
		231	274	105	213	251	218	182
	LL-6	229	254	115	215	223	221	174
		231	258	125	215	223	221	174
	LL-7	227	250	121	231	223	218	174
		231	254	123	235	223	222	180
	LL-8	231	260	105	215	229	218	174
		231	266	111	215	245	222	178
	LL-9	231	264	119	?	229	218	174
		231	264	119	?	249	220	182
	LL-10	229	256	113	215	225	218	174
		231	258	113	215	229	222	178
	LL-11	231	266	109	?	221	220	174
		231	270	119	?	229	222	180
	LL-12	231	262	103	219	227	220	174
		231	262	103	219	243	222	174
	LL-13	231	258	105	?	225	218	178
		231	266	119	?	225	222	180

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Sandy	H-1	229	254	111	219	227	220	174
River, WV		231	276	117	219	227	220	176
	H-2	231	254	107	215	229	220	174
		231	258	115	219	229	220	174
	H-3	233	248	111	219	227	222	174
		233	258	117	219	227	222	176
	H-4	231	244	115	227	219	220	174
		233	254	115	227	229	224	176
	H-5	229	254	115	213	229	220	174
		231	258	119	213	229	222	176
	H-6	231	244	111	207	227	220	176
		231	244	115	215	227	220	176
	H-7	229	254	115	213	229	220	174
		231	254	121	213	229	220	176
	H-8	229	244	115	219	219	220	174
		231	244	117	219	219	220	176
	H-9	231	248	115	221	225	220	174
		231	258	115	221	227	221	176
	H-10	229	254	105	215	219	220	174
		231	254	115	221	227	220	174
	H-11	229	254	117	219	229	220	174
		229	254	117	219	229	222	176
	H-12	231	254	107	215	225	220	174
		231	258	115	223	225	220	176
	H-13	229	248	115	219	229	220	174
		231	276	123	227	229	220	176
	H-14	231	248	117	221	223	220	174
		231	276	117	221	223	220	176
	H-15	229	244	115	221	227	220	176
		231	248	115	227	227	222	176
	H-17	229	254	115	225	229	218	174
		231	258	121	225	229	222	176
	H-18	229	256	119	215	225	220	176
		231	256	129	219	229	220	176
	H-19	231	244	115	215	233	220	176
		231	248	121	215	233	220	176
	H-20	231	254	111	?	223	220	174
		231	258	115	?	223	222	174

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Pennington	L-1	231	264	111	219	237	220	176
Creek, OK		231	264	111	219	237	220	176
	L-2	231	264	111	227	229	220	176
		231	264	111	227	237	220	176
	L-3	231	254	107	219	229	220	176
		231	264	115	219	229	220	176
	L-4	231	254	109	219	235	220	176
		231	264	119	227	235	220	176
	L-5	229	254	115	219	229	220	176
		231	264	115	219	233	220	176
	L-6	231	254	109	227	229	220	176
		231	264	109	227	237	220	176
	L-7	229	254	111	225	233	220	176
		229	264	111	225	233	220	176
	L-8	231	254	103	225	229	220	176
		231	264	103	225	245	220	176
	L-9	231	254	109	219	237	220	176
		231	254	109	219	237	220	176
	L-10	229	264	115	219	227	220	176
		229	264	121	227	237	220	176

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Nueces	N-1	231	244	113	225	227	220	176
River, TX		231	244	115	225	229	220	176
	N-2	227	244	115	221	229	220	176
		229	244	115	221	233	220	176
	N-3	227	244	113	219	233	220	176
		231	244	115	221	233	220	176
	N-4	227	244	113	221	233	220	176
		229	244	115	221	233	220	176
	N-5	229	244	115	219	233	220	176
		229	244	121	225	233	220	176

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Johnson	P-1	231	244	115	221	233	220	176
Creek, TX		231	244	115	221	233	220	176
	P-2	227	244	115	?	233	220	176
		229	244	115	?	235	220	176
	P-3	229	244	115	219	233	220	176
		231	244	115	221	233	220	176
	P-4	227	244	115	?	233	220	176
		231	244	115	?	233	220	176
	P-5	229	244	113	219	233	220	176
		231	244	113	219	233	220	176
	P-6	231	244	115	?	233	220	176
		231	244	115	?	235	220	176
	P-7	229	244	113	219	233	220	176
		231	244	113	219	233	220	176
	P-8	227	248	115	219	233	220	176
		229	248	121	219	233	220	176
	P-9	229	244	115	219	233	220	176
		231	244	121	219	233	220	176
	P-10	231	244	115	219	233	220	176
		231	244	115	219	233	220	176
	P-11	231	244	115	219	233	220	176
		231	244	115	219	233	220	176

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Bois D'Arc	Q-1	231	244	115	219	235	220	176
Creek, OK		231	264	109	219	235	220	176
	Q-2	231	264	109	219	237	220	176
		231	264	113	219	245	220	176
	Q-3	231	254	111	219	245	220	176
		231	254	123	219	245	220	176
	Q-4	231	254	127	219	235	220	176
		231	264	127	219	245	220	176
	Q-5	231	254	115	229	235	220	176
		231	264	115	229	235	220	176
	Q-6	231	254	111	219	229	220	176
		231	264	111	221	245	220	176
	Q-7	231	254	113	219	237	220	176
		231	264	127	219	245	220	176
	Q-8	231	254	113	229	229	220	176
		231	254	113	229	235	220	176
	Q-9	231	254	115	219	231	220	176
		231	254	115	219	237	220	176
	Q-10	231	254	113	219	229	220	176
		231	264	113	219	237	220	176
	Q-11	231	264	111	219	237	220	176
		231	264	115	219	237	220	176

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Pecos	R-1	233	254	109	211	229	218	178
River, NM		235	254	127	211	229	218	178
	R-2	229	254	109	217	251	218	178
		235	258	121	217	251	224	180
	R-3	229	258	109	217	229	218	178
		235	266	109	219	253	220	178
	R-4	229	254	109	211	251	218	178
		229	262	121	217	251	222	180
	R-5	229	260	113	217	231	220	178
		231	266	121	217	233	222	182
	R-6	231	254	109	211	229	218	174
		231	256	121	217	253	220	178
	R-7	231	258	107	211	229	218	178
		233	262	109	211	233	220	178
	R-8	231	256	109	215	233	218	174
		231	262	117	217	255	222	178
	R-9	233	260	109	211	229	218	178
		233	262	123	217	253	218	178
	R-10	229	274	109	215	251	220	180
		233	274	113	215	251	220	180
	R-11	231	254	113	211	233	218	174
		231	256	121	217	251	220	178
	R-12	231	274	113	217	233	218	174
		231	286	121	221	233	220	180
	R-13	233	254	121	219	233	218	178
		233	260	121	219	251	218	178
	R-14	229	256	109	217	229	218	174
		231	262	121	217	251	218	178
	R-15	233	262	107	211	233	218	178
		233	262	123	217	233	220	178
	R-16	235	254	107	217	229	222	174
		235	256	121	217	253	224	180
	R-17	231	254	109	217	253	218	180
		231	254	121	217	253	220	180
	R-18	231	254	113	211	233	218	178
		231	254	121	217	251	220	180
	R-19	229	254	121	211	233	220	174
		229	254	123	211	245	222	178

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Sandy	S-1	231	260	113	221	225	220	174
Creek, OK		231	264	127	229	243	220	174
	S-2	231	258	107	215	245	218	174
		235	260	117	217	253	220	174
	S-3	231	250	109	219	229	220	174
		231	260	121	221	255	222	178
	S-4	231	254	105	223	235	220	174
		231	256	121	223	257	222	180
	S-5	229	262	107	217	225	218	178
		233	264	107	217	229	220	180
	S-6	229	270	117	219	243	220	176
		229	272	121	219	251	220	180
	S-7	231	266	107	?	229	222	176
		231	270	121	?	229	222	178
	S-8	229	254	121	219	253	218	174
		231	258	121	223	253	220	178
	S-9	229	250	107	215	249	220	174
		231	258	115	215	249	222	178
	S-10	231	260	105	221	223	220	178
		235	260	117	221	255	220	178
	S-11	229	258	111	217	229	220	178
		231	260	115	217	253	222	178

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Turkey	T-1	229	268	115	219	229	220	178
Creek, OK		229	272	115	219	253	220	178
	T-2	229	260	111	215	245	224	178
		231	270	115	215	245	224	180
	T-3	229	260	113	221	229	218	178
		231	264	113	223	251	218	178
	T-4	229	258	107	221	235	222	178
		231	264	111	227	243	224	182
	T-5	231	258	115	221	251	218	178
		231	264	115	225	251	222	178
	T-6	229	252	119	213	235	220	178
		231	260	121	213	235	220	178
	T-7	231	258	107	215	229	220	174
		231	286	119	223	253	222	178
	T-8	231	264	111	215	223	220	174
		231	286	119	219	229	222	182
	T-9	231	260	115	219	229	218	176
		231	270	115	219	245	218	178
	T-10	231	258	109	229	223	222	174
		233	276	109	229	229	222	178
	T-11	229	260	105	221	235	218	178
		231	260	119	221	235	220	178
	T-12	229	262	111	213	229	218	178
		229	264	115	213	235	222	182
	T-13	231	254	121	215	253	218	176
		231	260	125	221	253	218	178
	T-14	229	254	109	219	223	220	174
		237	278	109	223	235	222	174
	T-15	229	274	107	221	235	220	174
		231	294	115	221	249	222	180

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Cedar	Y-1	231	258	115	221	223	220	174
Creek, IA		231	258	115	221	227	220	174
	Y-3	231	254	107	?	227	220	174
		231	264	107	?	239	222	178
	Y-4	231	250	105	215	229	220	174
		231	258	105	217	239	220	174
	Y-5	231	?	115	223	223	220	174
		231	?	123	223	235	220	174
	Y-8	229	256	115	219	227	220	178
		231	264	115	221	227	220	178
	Y-9	231	264	115	215	227	220	174
		231	264	115	215	241	222	174
	Y-10	231	258	103	215	233	220	174
		231	264	103	215	235	220	178
	Y-11	?	254	115	223	225	220	178
		?	264	115	231	227	222	178
	Y-12	231	258	103	219	223	220	174
		231	276	103	223	227	222	178
	Y-13	231	254	103	215	229	220	174
		231	262	117	225	255	222	174
	Y-14	231	264	107	215	227	220	174
		231	266	107	221	239	222	178

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Platte	Z-1	227	258	105	213	229	220	174
River, NE		227	260	109	213	229	222	174
	Z-2	227	262	109	213	229	220	174
		231	264	111	213	229	220	174
	Z-3	231	258	103	213	229	220	174
		231	258	105	229	231	220	174
	Z-4	227	258	109	213	229	220	174
		231	266	109	231	231	220	174
	Z-5	?	258	103	?	231	222	174
		?	266	103	?	231	222	174
	Z-6	227	256	109	213	229	220	174
		227	258	109	231	231	220	174
	Z-7	231	252	103	213	229	220	174
		231	262	113	213	229	222	174
	Z-8	227	256	109	230	229	220	174
		231	258	115	230	239	220	174
	Z-9	231	256	109	213	229	220	174
		231	258	109	213	229	220	174
	Z-10	227	258	109	213	229	220	174
		231	270	109	213	231	222	174
	Z-11	227	258	111	213	229	220	174
		227	258	111	213	233	220	174
	Z-12	227	262	109	230	229	220	174
		231	264	109	238	231	220	174
	Z-13	231	258	103	213	229	220	174
		231	262	109	227	229	222	174
	Z-14	227	260	103	233	229	220	174
		231	268	109	233	231	222	174
	Z-15	231	262	103	213	229	220	174
		231	262	109	227	231	222	174
	Z-16	227	258	109	213	229	220	174
		227	262	109	213	229	220	174
	Z-17	231	256	109	223	231	220	174
		231	262	109	229	231	220	174
	Z-18	227	258	113	213	231	220	174
		227	262	109	213	231	220	174
	Z-19	227	258	109	213	229	220	174
		231	266	111	213	229	220	174
	Z-20	227	256	105	213	229	222	174
		227	256	109	233	229	222	174

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Six Mile	AA-2	231	250	103	219	223	220	174
Creek, SD		231	262	113	219	229	222	174
	AA-3	229	250	115	219	223	220	174
		231	256	115	223	229	220	174
	AA-4	227	250	115	233	227	220	174
		231	258	115	233	239	220	174
	AA-5	227	250	115	225	227	220	174
		231	266	115	235	239	222	176
	AA-6	227	250	113	221	225	220	174
		231	250	115	221	225	220	174
	AA-7	229	256	103	221	235	220	174
		229	258	115	237	239	222	174
	AA-8	229	258	115	221	239	220	174
		231	286	115	221	239	222	178
	AA-9	229	282	115	221	227	220	174
		231	286	115	221	227	222	174
	AA-10	229	256	115	225	223	220	174
		229	262	115	237	229	220	174
	AA-11	231	266	115	221	227	220	174
		231	266	115	225	233	220	174
	AA-12	231	250	115	233	229	220	174
		231	256	115	233	229	220	174
	AA-13	229	250	105	221	223	222	174
		231	260	115	233	239	222	174
	AA-14	229	260	115	219	227	222	174
		229	266	115	219	239	222	174
	AA-15	231	250	107	221	227	220	174
		231	256	107	221	227	222	174
	AA-16	229	262	113	221	233	220	174
		229	264	115	221	233	220	174
	AA-17	229	262	115	215	229	220	174
		231	262	117	221	239	222	174
	AA-18	231	250	115	213	239	220	174
		231	266	117	235	239	222	174
	AA-19	229	258	107	221	233	220	174
		231	266	107	225	233	220	174
	AA-20	227	262	115	225	223	220	174
		231	266	115	233	229	220	174

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Niobrara	BB-1	231	256	109	213	229	220	174
River, NE		231	270	109	237	229	220	174
	BB-3	227	266	111	213	229	220	174
		231	268	113	233	229	220	174
	BB-4	227	258	105	213	229	220	174
		231	262	113	213	231	220	174
	BB-6	227	256	103	213	229	220	174
		227	258	109	213	229	222	174
	BB-7	227	258	103	213	229	220	174
		227	262	109	227	229	220	174
	BB-10	227	256	103	207	229	220	174
		231	262	111	211	229	220	174
	BB-12	227	256	103	213	227	220	174
		227	262	113	213	229	220	174
	BB-13	227	256	101	227	227	220	174
		231	258	103	227	227	220	174
	BB-14	?	256	105	213	229	220	174
		?	258	109	239	231	222	174

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
North	CC-3	227	256	103	211	229	220	174
Platte		231	258	109	225	229	220	174
River, NE	CC-4	227	256	103	213	235	220	174
		227	260	113	213	239	220	178
	CC-8	227	258	103	207	229	220	174
		231	262	109	221	231	220	174
	CC-10	?	260	103	213	229	220	174
		?	266	109	227	229	220	178
	CC-12	?	258	111	213	225	220	174
		?	258	111	227	225	220	174
	CC-13	231	252	103	209	229	220	174
		231	258	109	225	229	220	174
	CC-14	227	258	111	215	229	220	174
		227	258	111	225	233	220	174
	CC-15	227	256	109	225	229	220	174
		231	258	113	227	233	220	174
	CC-16	227	256	103	225	227	220	174
		231	258	109	237	227	220	174
	CC-18	227	250	109	225	227	220	174
		231	258	115	231	239	220	174
	CC-19	227	250	109	225	229	220	174
		227	262	113	225	229	222	174

? - indicates missing data

Population	Sample Code	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5	Locus 6	Locus 7
Smoky	EE-1	227	268	107	213	229	222	174
Hill River,		233	270	109	215	233	222	174
KS	EE-2	227	266	105	207	223	222	174
		231	268	105	207	229	222	174
	EE-3	227	258	111	219	229	222	174
		231	260	111	223	229	222	174
	EE-4	229	264	107	213	229	222	174
		231	264	109	221	229	222	174
	EE-6	231	258	109	215	225	220	174
		231	264	123	217	229	222	176
	EE-7	231	258	109	217	221	220	174
		231	274	109	217	225	222	174
	EE-8	231	254	103	215	221	220	174
		231	264	103	221	225	222	174
	EE-9	227	270	107	211	223	222	174
		227	272	107	211	223	222	180
	EE-10	227	266	103	215	229	220	174
		231	266	103	227	229	220	174
	EE-11	231	264	115	229	231	222	174
		231	284	115	229	231	222	174
	EE-12	227	252	105	221	229	218	174
		231	292	113	221	233	222	174
	EE-13	227	266	109	213	233	222	174
		231	272	109	229	233	222	178
	EE-14	231	260	107	215	235	218	174
		231	284	119	237	235	222	174
	EE-15	231	256	97	229	229	222	174
		233	272	113	229	229	222	174
	EE-16	231	266	107	217	233	220	174
		231	276	107	221	233	222	174
	EE-17	231	260	103	213	233	222	174
		231	260	107	213	233	222	174
	EE-18	233	258	109	217	225	220	174
		239	272	111	217	229	222	174
	EE-19	231	256	103	225	229	220	174
		231	258	103	225	231	228	174
	EE-20	231	260	107	229	223	218	174
		231	262	109	229	229	220	174

Appendix 5
Morphological measurements made on *N. stramineus* specimens (given in mm).

	Standard Length	Dorsal Head Length (10-1)	Ventral Head Length (10-9)	Head Depth (1-9)	Occiput to Pectoral Origin (1-8)
1 - Lake Creek, IA	50.71	9.81	8.04	7.85	7.62
1 - Lake Creek, IA	47.22	9.37	6.52	6.54	7.39
1 - Lake Creek, IA	48.37	10.03	8.17	7.86	7.37
1 - Lake Creek, IA	49.06	10.40	8.39	7.26	7.83
1 - Lake Creek, IA	48.29	10.32	7.33	7.94	7.58
1 - Lake Creek, IA	47.77	10.65	6.96	8.47	7.47
1 - Lake Creek, IA	42.82	8.88	6.08	6.31	6.77
1 - Lake Creek, IA	41.26	8.58	6.52	6.07	6.03
1 - Lake Creek, IA	44.77	8.84	6.70	7.01	7.83
1 - Lake Creek, IA	52.65	10.54	7.33	8.30	8.20
2 - Platte River, NE	37.87	8.08	6.18	6.20	5.57
2 - Platte River, NE	39.50	8.35	6.21	6.21	5.68
2 - Platte River, NE	40.63	8.32	6.48	6.09	5.08
2 - Platte River, NE	40.29	8.15	6.33	6.33	5.85
2 - Platte River, NE	42.17	8.71	6.29	6.30	5.53
2 - Platte River, NE	44.83	8.87	6.83	6.51	6.42
2 - Platte River, NE	40.17	8.27	6.50	6.23	5.67
2 - Platte River, NE	38.92	7.81	5.74	5.83	5.70
2 - Platte River, NE	41.92	9.07	7.48	6.54	6.44
3 - Kansas River, KS	34.08	6.96	3.79	5.60	4.96
3 - Kansas River, KS	33.06	6.98	5.13	5.32	5.25
3 - Kansas River, KS	31.80	6.79	4.14	5.55	4.85
3 - Kansas River, KS	32.27	7.25	5.06	5.74	5.03
3 - Kansas River, KS	33.04	6.91	4.23	4.62	4.82
3 - Kansas River, KS	31.97	6.19	5.00	4.66	4.93
3 - Kansas River, KS	30.82	6.55	4.30	4.41	4.36
3 - Kansas River, KS	31.01	6.26	5.06	4.73	4.28
3 - Kansas River, KS	28.35	6.45	4.21	4.57	3.99
4 - Arkansas River, KS	45.34	9.90	7.07	7.57	8.02
4 - Arkansas River, KS	50.79	11.13	9.02	8.42	9.10
4 - Arkansas River, KS	44.94	9.53	8.03	7.37	7.82
4 - Arkansas River, KS	46.83	10.02	7.15	7.58	7.72
4 - Arkansas River, KS	47.65	10.65	8.08	7.08	7.60
4 - Arkansas River, KS	47.87	10.21	7.13	7.74	7.58
4 - Arkansas River, KS	51.12	10.52	8.00	8.30	8.67
4 - Arkansas River, KS	46.66	10.13	6.24	7.68	7.97
4 - Arkansas River, KS	43.68	9.99	7.35	7.43	7.50
4 - Arkansas River, KS	41.99	8.95	6.21	6.33	5.83
4 - Arkansas River, KS	47.04	9.16	8.19	8.03	8.32
4 - Arkansas River, KS	48.13	10.07	7.34	8.05	8.14
4 - Arkansas River, KS	44.48	9.25	7.15	7.36	7.91
5 - Pecos River, NM	43.16	9.27	6.30	7.01	6.99
5 - Pecos River, NM	47.89	9.98	7.91	8.58	8.00
5 - Pecos River, NM	43.76	9.27	6.95	7.61	7.85
5 - Pecos River, NM	44.67	9.62	7.74	7.81	7.74

Population	Standard Length	Dorsal Head Length (10-1)	Ventral Head Length (10-9)	Head Depth (1-9)	Occiput to Pectoral Origin (1-8)
5 - Pecos River, NM	40.46	8.99	6.67	7.00	6.49
5 - Pecos River, NM	52.06	10.84	7.92	8.71	8.97
5 - Pecos River, NM	43.24	9.21	7.84	7.58	7.77
5 - Pecos River, NM	40.75	8.55	6.45	6.28	6.44
5 - Pecos River, NM	43.16	8.71	6.68	7.29	6.82
5 - Pecos River, NM	44.38	9.29	7.16	7.35	7.37
6 - Conchas River, NM	41.80	8.61	7.85	7.09	7.49
6 - Conchas River, NM	40.64	7.76	7.17	6.44	6.62
6 - Conchas River, NM	41.28	8.67	7.75	7.02	7.47
6 - Conchas River, NM	41.33	8.46	7.37	7.18	7.50
6 - Conchas River, NM	35.67	7.19	6.36	6.08	6.45
6 - Conchas River, NM	41.72	8.23	7.25	7.16	7.50
6 - Conchas River, NM	38.82	7.59	6.76	6.45	6.91
6 - Conchas River, NM	38.12	7.91	6.38	6.71	6.86
6 - Conchas River, NM	35.68	7.07	5.99	5.78	6.23
6 - Conchas River, NM	42.93	8.69	6.81	7.15	7.72
7 - Sandy Creek, TX	47.08	10.12	7.86	7.66	8.27
7 - Sandy Creek, TX	50.30	10.69	7.65	8.40	8.58
7 - Sandy Creek, TX	48.28	10.39	7.30	8.14	8.43
7 - Sandy Creek, TX	47.01	10.30	8.01	7.85	8.83
7 - Sandy Creek, TX	47.25	9.77	7.49	7.74	7.70
7 - Sandy Creek, TX	40.72	8.28	6.46	6.28	6.27
7 - Sandy Creek, TX	47.76	10.07	7.70	8.10	7.56
7 - Sandy Creek, TX	46.39	9.65	7.39	7.24	7.64
7 - Sandy Creek, TX	41.79	9.09	6.81	6.87	6.95
7 - Sandy Creek, TX	46.51	9.61	7.06	7.46	7.70
7 - Sandy Creek, TX	36.11	7.95	5.98	5.55	5.93
8 - Frio River, TX	47.50	10.23	8.50	8.22	7.42
8 - Frio River, TX	35.25	8.41	6.32	5.88	5.39
8 - Frio River, TX	38.25	8.77	7.27	6.41	6.54
8 - Frio River, TX	39.25	9.04	7.54	6.66	6.86
8 - Frio River, TX	38.28	8.37	7.00	6.27	5.92
8 - Frio River, TX	43.47	9.99	8.11	7.12	7.08
8 - Frio River, TX	40.39	9.10	6.73	6.01	6.31
8 - Frio River, TX	39.96	8.86	7.14	6.67	6.62
8 - Frio River, TX	39.58	9.49	7.17	6.94	6.31
8 - Frio River, TX	40.91	9.53	7.64	6.81	6.55
9 - Clear Boggy Creek, OK	52.74	11.38	8.31	8.54	8.94
9 - Clear Boggy Creek, OK	51.17	10.83	7.59	8.52	8.90
9 - Clear Boggy Creek, OK	38.16	8.25	6.74	6.08	6.10
9 - Clear Boggy Creek, OK	34.11	6.77	5.36	5.36	5.40
9 - Clear Boggy Creek, OK	41.04	8.69	6.56	6.77	6.57
9 - Clear Boggy Creek, OK	37.43	8.07	4.72	5.85	6.32
9 - Clear Boggy Creek, OK	40.71	8.59	6.72	6.60	6.35
9 - Clear Boggy Creek, OK	38.10	7.60	4.71	5.78	6.40
9 - Clear Boggy Creek, OK	34.78	7.59	5.60	5.46	5.64
9 - Clear Boggy Creek, OK	32.54	7.24	5.29	5.15	5.06

Population	Occiput to Pelvic Origin (1-7)	Predorsal Length (1-2)	Dorsal Origin to Isthmus (2-9)	Dorsal to Pectoral Origin (2-8)	Predorsal depth (2-7)
1 - Lake Creek, IA	17.85	16.92	19.51	14.79	11.91
1 - Lake Creek, IA	17.70	15.12	18.92	13.63	10.63
1 - Lake Creek, IA	17.86	16.15	18.73	14.61	10.66
1 - Lake Creek, IA	18.31	15.97	20.37	14.40	10.79
1 - Lake Creek, IA	16.84	15.60	18.80	12.94	10.04
1 - Lake Creek, IA	18.38	15.16	18.71	13.40	11.56
1 - Lake Creek, IA	16.14	13.72	18.06	11.56	9.33
1 - Lake Creek, IA	15.88	13.80	16.61	11.85	9.00
1 - Lake Creek, IA	17.01	14.07	17.85	12.13	10.99
1 - Lake Creek, IA	19.70	17.99	19.86	14.72	11.28
2 - Platte River, NE	14.45	11.05	14.10	10.48	7.63
2 - Platte River, NE	14.68	11.53	14.34	11.30	8.03
2 - Platte River, NE	14.11	12.76	15.42	11.34	8.32
2 - Platte River, NE	13.99	12.33	15.76	11.40	7.53
2 - Platte River, NE	14.47	13.81	15.57	11.05	7.93
2 - Platte River, NE	15.53	12.69	17.67	12.03	9.14
2 - Platte River, NE	14.30	12.57	16.01	11.87	7.91
2 - Platte River, NE	13.46	11.29	14.43	10.67	7.53
2 - Platte River, NE	14.64	12.17	15.61	11.44	7.33
3 - Kansas River, KS	12.12	10.17	15.22	9.39	6.82
3 - Kansas River, KS	12.76	9.66	14.04	10.05	6.59
3 - Kansas River, KS	12.67	10.07	12.99	8.99	6.64
3 - Kansas River, KS	13.25	10.88	13.40	9.29	6.84
3 - Kansas River, KS	11.29	10.15	12.38	8.48	6.09
3 - Kansas River, KS	12.14	9.73	14.25	8.65	6.65
3 - Kansas River, KS	11.18	9.44	11.97	7.60	6.44
3 - Kansas River, KS	11.43	9.69	12.20	8.20	5.79
3 - Kansas River, KS	10.27	8.84	11.66	8.16	6.11
4 - Arkansas River, KS	17.89	14.07	15.41	12.70	9.70
4 - Arkansas River, KS	17.95	16.38	20.13	14.52	10.55
4 - Arkansas River, KS	17.11	12.76	15.77	12.85	10.19
4 - Arkansas River, KS	17.48	13.66	17.74	12.99	9.77
4 - Arkansas River, KS	16.42	14.15	17.66	12.26	10.37
4 - Arkansas River, KS	16.60	14.37	16.55	12.72	8.87
4 - Arkansas River, KS	19.98	14.38	18.58	14.14	10.51
4 - Arkansas River, KS	16.06	13.74	19.78	11.93	9.81
4 - Arkansas River, KS	15.97	14.04	16.79	12.39	9.30
4 - Arkansas River, KS	13.23	13.89	15.78	11.26	8.14
4 - Arkansas River, KS	17.90	13.75	18.52	11.82	9.94
4 - Arkansas River, KS	18.34	14.81	20.23	13.29	10.39
4 - Arkansas River, KS	18.10	13.94	17.64	11.77	10.72
5 - Pecos River, NM	16.23	12.66	16.87	11.68	9.31
5 - Pecos River, NM	17.97	14.52	19.39	13.24	9.98
5 - Pecos River, NM	15.90	13.23	17.73	12.09	9.66
5 - Pecos River, NM	17.66	14.11	19.43	12.90	10.11

Population	Occiput to Pelvic Origin (1-7)	Predorsal Length (1-2)	Dorsal Origin to Isthmus (2-9)	Dorsal to Pectoral Origin (2-8)	Predorsal depth (2-7)
5 - Pecos River, NM	14.80	12.12	16.07	11.30	8.28
5 - Pecos River, NM	19.34	16.83	21.07	14.23	10.97
5 - Pecos River, NM	15.82	13.66	17.70	11.88	9.98
5 - Pecos River, NM	14.78	11.57	14.97	10.17	8.60
5 - Pecos River, NM	15.18	12.88	16.85	11.92	9.46
5 - Pecos River, NM	15.98	13.56	17.05	12.25	9.69
6 - Canadian River, NM	16.43	12.73	16.30	12.04	10.44
6 - Canadian River, NM	15.05	12.83	15.16	11.26	9.83
6 - Canadian River, NM	15.95	12.52	16.40	11.63	10.08
6 - Canadian River, NM	15.95	11.85	16.18	11.45	10.53
6 - Canadian River, NM	13.90	10.57	14.98	10.60	8.94
6 - Canadian River, NM	16.15	12.39	15.73	11.38	10.14
6 - Canadian River, NM	14.95	12.28	15.65	11.58	9.96
6 - Canadian River, NM	15.32	11.49	14.86	10.35	9.26
6 - Canadian River, NM	14.11	10.75	13.64	9.93	8.75
6 - Canadian River, NM	16.15	13.09	17.01	11.96	9.87
7 - Sandy Creek, TX	18.44	13.72	17.17	11.70	10.08
7 - Sandy Creek, TX	18.14	13.84	17.62	12.04	11.65
7 - Sandy Creek, TX	19.86	15.68	20.16	15.37	12.25
7 - Sandy Creek, TX	19.30	13.74	18.82	13.47	10.79
7 - Sandy Creek, TX	17.91	14.45	18.02	12.71	10.91
7 - Sandy Creek, TX	16.08	11.64	15.12	12.02	8.88
7 - Sandy Creek, TX	19.26	14.91	19.20	13.80	11.30
7 - Sandy Creek, TX	18.54	14.81	17.47	11.50	9.76
7 - Sandy Creek, TX	15.97	12.39	15.93	12.16	8.97
7 - Sandy Creek, TX	18.63	14.48	17.94	12.30	10.08
7 - Sandy Creek, TX	14.15	12.42	13.46	9.35	7.46
8 - Frio River, TX	16.92	13.23	17.59	12.99	10.59
8 - Frio River, TX	12.84	10.16	13.85	9.99	7.82
8 - Frio River, TX	14.11	11.00	15.44	10.81	8.81
8 - Frio River, TX	15.61	11.38	15.27	10.54	8.50
8 - Frio River, TX	13.79	10.49	15.13	11.13	8.27
8 - Frio River, TX	15.85	12.46	16.72	11.47	9.14
8 - Frio River, TX	14.91	11.62	15.13	10.08	8.70
8 - Frio River, TX	15.15	11.41	15.16	9.56	8.43
8 - Frio River, TX	15.07	11.57	14.14	10.64	8.55
8 - Frio River, TX	15.13	12.05	14.89	11.30	8.90
9 - Clear Boogy Creek, OK	20.51	17.44	20.94	14.77	10.22
9 - Clear Boogy Creek, OK	19.86	16.44	20.61	14.34	9.68
9 - Clear Boogy Creek, OK	14.39	11.78	15.18	10.61	7.68
9 - Clear Boogy Creek, OK	11.96	9.75	12.52	9.64	6.38
9 - Clear Boogy Creek, OK	15.70	12.29	15.96	12.15	9.32
9 - Clear Boogy Creek, OK	13.82	11.73	14.98	10.49	8.40
9 - Clear Boogy Creek, OK	14.99	11.27	15.37	10.57	8.57
9 - Clear Boogy Creek, OK	14.81	11.46	15.13	10.52	8.24
9 - Clear Boogy Creek, OK	12.69	10.88	13.92	9.93	7.64
9 - Clear Boogy Creek, OK	11.78	9.06	12.63	8.18	7.36

Population	Dorsal to Anal Origin (2-6)	Dorsal Fin Base (2-3)	Posterior Dorsal to Pectoral origin (3-7)	Post-dorsal Depth (3-6)	Dorsal Peduncle Length (3-4)
1 - Lake Creek, IA	14.58	6.02	18.39	9.15	20.58
1 - Lake Creek, IA	12.66	5.22	16.94	8.62	18.08
1 - Lake Creek, IA	12.96	6.42	17.33	8.17	18.35
1 - Lake Creek, IA	12.92	6.21	18.15	9.28	16.68
1 - Lake Creek, IA	13.48	5.47	16.63	8.61	18.57
1 - Lake Creek, IA	14.05	6.03	16.68	9.33	17.72
1 - Lake Creek, IA	12.06	5.62	14.20	6.79	15.68
1 - Lake Creek, IA	12.40	5.20	15.72	7.57	15.37
1 - Lake Creek, IA	12.06	5.43	16.04	8.52	17.12
1 - Lake Creek, IA	14.22	6.73	19.40	9.07	20.23
2 - Platte River, NE	9.63	3.91	13.34	6.57	15.87
2 - Platte River, NE	10.10	4.54	14.86	6.93	15.66
2 - Platte River, NE	10.61	4.53	15.25	7.21	16.14
2 - Platte River, NE	10.66	4.79	14.99	6.27	16.44
2 - Platte River, NE	9.50	5.31	15.72	7.27	16.77
2 - Platte River, NE	11.79	5.93	17.16	7.90	17.67
2 - Platte River, NE	9.98	4.79	15.23	7.11	15.20
2 - Platte River, NE	9.12	4.19	13.86	6.27	15.11
2 - Platte River, NE	9.31	4.68	14.35	5.54	16.83
3 - Kansas River, KS	9.21	4.14	11.80	6.07	15.34
3 - Kansas River, KS	9.39	4.54	12.81	5.76	13.82
3 - Kansas River, KS	9.29	4.39	10.62	5.45	12.40
3 - Kansas River, KS	8.68	4.11	12.32	5.15	13.95
3 - Kansas River, KS	7.55	4.01	12.87	5.54	13.69
3 - Kansas River, KS	8.28	4.06	11.61	4.75	13.18
3 - Kansas River, KS	8.04	3.89	10.60	4.69	12.85
3 - Kansas River, KS	7.41	4.64	11.13	5.48	12.60
3 - Kansas River, KS	7.34	4.02	10.08	4.59	11.12
4 - Arkansas River, KS	13.73	6.54	16.81	6.77	17.16
4 - Arkansas River, KS	14.07	8.05	18.38	8.33	19.04
4 - Arkansas River, KS	11.47	5.60	17.70	8.01	15.09
4 - Arkansas River, KS	12.81	6.26	17.52	7.55	19.87
4 - Arkansas River, KS	12.65	5.60	15.62	9.09	20.10
4 - Arkansas River, KS	11.22	5.82	16.61	7.28	18.50
4 - Arkansas River, KS	13.67	6.81	18.32	8.95	19.11
4 - Arkansas River, KS	13.44	6.27	16.23	7.34	18.12
4 - Arkansas River, KS	12.68	5.02	15.50	8.33	16.22
4 - Arkansas River, KS	11.90	5.95	15.94	6.17	15.71
4 - Arkansas River, KS	13.27	6.83	16.00	8.07	19.42
4 - Arkansas River, KS	12.73	5.60	17.69	8.45	18.85
4 - Arkansas River, KS	12.67	5.38	15.57	7.67	15.69
5 - Pecos River, NM	11.27	5.83	16.62	7.71	16.75
5 - Pecos River, NM	12.15	6.67	17.40	8.37	19.96
5 - Pecos River, NM	11.50	6.20	16.40	8.21	17.94
5 - Pecos River, NM	12.12	5.67	16.90	8.45	17.81

Population	Dorsal to Anal Origin (2-6)	Dorsal Fin Base (2-3)	Posterior Dorsal to Pectoral origin (3-7)	Post-dorsal Depth (3-6)	Dorsal Peduncle Length (3-4)
5 - Pecos River, NM	10.68	5.64	14.51	7.29	15.77
5 - Pecos River, NM	13.22	6.82	19.01	9.04	21.50
5 - Pecos River, NM	11.55	5.38	16.04	8.52	18.16
5 - Pecos River, NM	11.40	4.84	14.48	7.64	17.33
5 - Pecos River, NM	11.86	5.67	16.28	8.34	17.39
5 - Pecos River, NM	12.18	6.67	16.59	8.39	17.67
6 - Canadian River, NM	12.79	5.45	16.44	8.73	16.38
6 - Canadian River, NM	11.78	5.83	14.94	7.66	15.68
6 - Canadian River, NM	13.01	5.64	15.38	8.78	16.09
6 - Canadian River, NM	13.76	5.76	15.10	8.63	17.21
6 - Canadian River, NM	10.53	4.81	13.37	6.56	13.25
6 - Canadian River, NM	12.94	5.24	14.95	8.48	17.67
6 - Canadian River, NM	11.75	4.84	15.37	7.94	15.20
6 - Canadian River, NM	11.51	5.21	14.14	7.35	15.07
6 - Canadian River, NM	10.14	4.31	13.13	6.79	14.10
6 - Canadian River, NM	12.91	5.12	15.01	9.13	16.85
7 - Sandy Creek, TX	14.04	6.17	16.14	8.04	18.33
7 - Sandy Creek, TX	14.54	6.77	17.46	9.25	20.10
7 - Sandy Creek, TX	14.50	6.29	17.83	9.05	19.03
7 - Sandy Creek, TX	12.90	6.11	17.40	7.97	18.81
7 - Sandy Creek, TX	14.13	5.71	15.57	8.31	19.13
7 - Sandy Creek, TX	11.69	5.26	15.26	7.75	16.34
7 - Sandy Creek, TX	13.39	6.06	16.97	8.76	17.91
7 - Sandy Creek, TX	12.15	5.35	15.78	7.88	18.89
7 - Sandy Creek, TX	11.84	5.20	14.97	7.31	16.95
7 - Sandy Creek, TX	12.84	5.61	16.37	7.97	19.29
7 - Sandy Creek, TX	9.17	4.04	12.41	5.88	13.61
8 - Frio River, TX	14.39	5.39	16.38	9.48	19.45
8 - Frio River, TX	10.36	4.06	12.97	7.29	14.16
8 - Frio River, TX	11.51	4.57	14.28	8.06	15.08
8 - Frio River, TX	11.22	4.71	14.38	8.05	15.54
8 - Frio River, TX	10.33	4.41	14.54	7.49	15.46
8 - Frio River, TX	12.53	5.17	15.51	9.21	17.80
8 - Frio River, TX	11.87	5.01	14.38	7.40	16.19
8 - Frio River, TX	12.04	4.76	13.30	7.99	16.17
8 - Frio River, TX	11.92	4.72	14.17	8.17	16.62
8 - Frio River, TX	12.29	4.92	14.69	8.09	16.14
9 - Clear Boogy Creek, OK	14.38	6.18	19.92	10.75	21.38
9 - Clear Boogy Creek, OK	14.15	6.96	19.60	9.43	19.51
9 - Clear Boogy Creek, OK	10.62	5.20	14.36	7.40	14.73
9 - Clear Boogy Creek, OK	9.56	4.22	12.14	6.03	13.65
9 - Clear Boogy Creek, OK	11.80	5.14	15.31	8.37	16.70
9 - Clear Boogy Creek, OK	11.41	4.49	13.00	7.24	15.84
9 - Clear Boogy Creek, OK	11.42	5.85	14.44	7.86	14.85
9 - Clear Boogy Creek, OK	10.71	4.78	13.08	6.98	15.80
9 - Clear Boogy Creek, OK	10.27	4.17	12.46	6.05	13.40
9 - Clear Boogy Creek, OK	9.58	3.85	11.94	5.83	13.04

Population	Ventral Peduncle Length (5-4)	Anal-fin Base (6-5)	Postpelvic Length (7-6)	Pectoral to Pelvic Origin (8-7)	Prepelvic Length (7-9)
1 - Lake Creek, IA	10.93	4.37	7.91	11.36	15.98
1 - Lake Creek, IA	11.39	4.27	8.58	11.85	17.63
1 - Lake Creek, IA	10.17	4.98	9.08	10.85	16.53
1 - Lake Creek, IA	10.26	4.81	9.91	10.43	16.59
1 - Lake Creek, IA	11.15	4.83	8.43	10.14	16.95
1 - Lake Creek, IA	10.67	4.64	8.19	11.15	17.99
1 - Lake Creek, IA	9.34	5.26	8.11	11.31	16.82
1 - Lake Creek, IA	9.27	3.79	8.51	9.10	15.08
1 - Lake Creek, IA	10.37	4.28	8.65	9.62	15.44
1 - Lake Creek, IA	11.41	4.90	9.96	11.76	18.12
2 - Platte River, NE	8.47	4.21	6.17	8.87	13.65
2 - Platte River, NE	8.93	4.01	6.61	9.19	14.35
2 - Platte River, NE	9.19	4.04	6.48	10.10	14.87
2 - Platte River, NE	9.58	4.24	6.46	9.28	13.76
2 - Platte River, NE	10.09	4.57	7.32	9.51	14.51
2 - Platte River, NE	11.20	4.57	8.18	10.08	14.84
2 - Platte River, NE	9.42	4.20	6.84	9.97	14.09
2 - Platte River, NE	9.68	4.33	6.09	9.41	13.74
2 - Platte River, NE	10.73	4.68	6.31	9.76	14.07
3 - Kansas River, KS	7.63	3.36	6.44	7.85	13.56
3 - Kansas River, KS	8.24	2.74	5.37	8.88	12.95
3 - Kansas River, KS	6.05	3.95	6.39	7.25	10.95
3 - Kansas River, KS	7.17	3.61	5.46	7.22	11.29
3 - Kansas River, KS	8.97	3.69	5.34	7.56	11.75
3 - Kansas River, KS	7.75	3.09	5.46	6.78	11.05
3 - Kansas River, KS	6.79	3.28	4.64	6.47	10.62
3 - Kansas River, KS	6.12	4.34	5.49	5.61	9.77
3 - Kansas River, KS	6.06	2.90	5.43	4.62	8.96
4 - Arkansas River, KS	10.58	4.74	8.60	10.73	17.81
4 - Arkansas River, KS	10.86	4.84	10.99	12.67	18.94
4 - Arkansas River, KS	11.13	4.59	8.82	9.86	17.88
4 - Arkansas River, KS	12.20	4.59	8.53	11.28	18.64
4 - Arkansas River, KS	15.47	4.86	6.75	9.99	17.40
4 - Arkansas River, KS	14.37	4.13	8.74	10.73	18.56
4 - Arkansas River, KS	15.30	4.72	10.12	12.34	18.67
4 - Arkansas River, KS	13.84	4.52	7.59	11.69	17.66
4 - Arkansas River, KS	12.03	4.17	9.11	10.45	17.09
4 - Arkansas River, KS	13.13	4.51	8.00	9.93	14.60
4 - Arkansas River, KS	14.49	4.98	7.55	9.08	16.18
4 - Arkansas River, KS	15.07	4.08	7.89	11.13	18.01
4 - Arkansas River, KS	13.90	4.47	7.03	12.22	17.26
5 - Pecos River, NM	10.63	4.79	6.67	9.14	14.88
5 - Pecos River, NM	12.31	5.23	7.87	10.08	16.71
5 - Pecos River, NM	10.46	4.52	6.46	8.58	14.64
5 - Pecos River, NM	10.68	4.79	7.36	9.65	15.08

Population	Ventral Peduncle Length (5-4)	Anal-fin Base (6-5)	Postpelvic Length (7-6)	Pectoral to Pelvic Origin (8-7)	Prepelvic Length (7-9)
5 - Pecos River, NM	10.26	4.64	6.29	8.48	13.02
5 - Pecos River, NM	12.86	5.87	7.46	11.01	18.06
5 - Pecos River, NM	11.13	4.77	6.98	9.73	15.43
5 - Pecos River, NM	11.08	4.47	5.94	8.47	14.19
5 - Pecos River, NM	10.33	4.83	6.83	9.71	14.70
5 - Pecos River, NM	10.85	5.29	7.04	9.91	14.96
6 - Canadian River, NM	9.25	4.33	7.29	9.51	14.77
6 - Canadian River, NM	10.05	4.16	6.91	9.62	13.93
6 - Canadian River, NM	10.05	4.42	6.75	8.99	13.75
6 - Canadian River, NM	9.37	4.36	7.20	9.26	13.95
6 - Canadian River, NM	8.40	3.91	6.29	8.66	12.15
6 - Canadian River, NM	10.36	4.34	7.30	9.42	13.37
6 - Canadian River, NM	8.76	3.98	6.57	9.44	13.39
6 - Canadian River, NM	8.62	4.10	6.29	8.67	13.16
6 - Canadian River, NM	8.78	3.63	5.49	8.26	12.22
6 - Canadian River, NM	9.76	4.28	7.78	9.39	14.52
7 - Sandy Creek, TX	12.02	5.33	9.08	10.17	15.81
7 - Sandy Creek, TX	15.67	5.50	8.83	10.57	17.11
7 - Sandy Creek, TX	12.16	5.60	9.13	12.99	19.08
7 - Sandy Creek, TX	11.88	4.14	7.83	11.91	17.86
7 - Sandy Creek, TX	11.46	4.90	8.59	10.73	16.57
7 - Sandy Creek, TX	9.70	3.42	7.23	10.53	14.86
7 - Sandy Creek, TX	12.25	4.41	7.75	12.24	17.60
7 - Sandy Creek, TX	12.18	3.76	7.24	10.24	16.12
7 - Sandy Creek, TX	10.31	3.57	6.07	10.56	14.15
7 - Sandy Creek, TX	11.80	3.76	7.87	10.62	14.94
7 - Sandy Creek, TX	7.64	2.77	6.90	8.79	12.07
8 - Frio River, TX	11.71	4.53	7.65	10.10	16.21
8 - Frio River, TX	8.58	3.36	5.64	8.15	11.10
8 - Frio River, TX	9.40	3.42	6.06	8.91	12.77
8 - Frio River, TX	9.86	3.59	6.47	8.94	12.99
8 - Frio River, TX	9.69	3.22	5.48	8.62	12.28
8 - Frio River, TX	10.53	3.94	7.26	9.99	14.39
8 - Frio River, TX	10.36	3.63	6.25	9.01	13.01
8 - Frio River, TX	9.59	3.26	6.72	8.77	13.28
8 - Frio River, TX	9.51	3.55	6.58	8.56	13.11
8 - Frio River, TX	9.71	3.69	6.62	9.45	14.82
9 - Clear Boogy Creek, OK	14.07	5.13	9.09	11.74	18.44
9 - Clear Boogy Creek, OK	13.23	5.18	8.19	10.70	18.15
9 - Clear Boogy Creek, OK	9.87	4.33	7.04	8.93	12.73
9 - Clear Boogy Creek, OK	9.40	2.82	5.66	7.94	12.16
9 - Clear Boogy Creek, OK	10.12	3.75	6.68	9.01	14.41
9 - Clear Boogy Creek, OK	9.60	3.08	6.62	7.64	13.82
9 - Clear Boogy Creek, OK	9.20	3.63	6.03	7.98	13.19
9 - Clear Boogy Creek, OK	9.50	3.94	6.39	8.55	14.23
9 - Clear Boogy Creek, OK	8.25	3.12	5.14	7.31	12.99
9 - Clear Boogy Creek, OK	9.34	3.27	5.08	7.37	12.09

Population	Head Width	Pectoral-fin Length	Pelvic-fin Length	Dorsal-fin Length (depressed)	Anal-fin Length (depressed)
1 - Lake Creek, IA	6.08	8.31	7.69	9.81	9.00
1 - Lake Creek, IA	5.49	8.31	6.65	9.41	7.51
1 - Lake Creek, IA	5.97	7.65	7.63	8.84	7.89
1 - Lake Creek, IA	6.01	8.67	8.11	9.78	8.63
1 - Lake Creek, IA	6.59	7.97	7.69	9.85	8.83
1 - Lake Creek, IA	6.00	7.79	8.09	9.86	7.79
1 - Lake Creek, IA	5.09	7.25	6.68	8.50	7.71
1 - Lake Creek, IA	5.16	6.43	6.64	9.37	6.60
1 - Lake Creek, IA	5.40	7.30	6.35	9.46	7.41
1 - Lake Creek, IA	6.30	8.44	7.44	10.84	7.74
2 - Platte River, NE	4.93	7.41	5.87	8.16	6.87
2 - Platte River, NE	4.87	8.53	6.28	9.13	6.92
2 - Platte River, NE	5.03	7.42	5.89	8.15	7.00
2 - Platte River, NE	4.82	8.03	6.29	8.74	7.15
2 - Platte River, NE	4.51	8.19	6.34	9.11	7.42
2 - Platte River, NE	5.17	8.54	6.92	9.75	7.96
2 - Platte River, NE	4.53	8.18	6.21	9.01	7.37
2 - Platte River, NE	4.56	7.28	5.91	8.98	7.03
2 - Platte River, NE	5.13	7.60	5.73	8.85	7.45
3 - Kansas River, KS	3.84	6.30	6.20	7.97	6.22
3 - Kansas River, KS	4.10	5.32	5.88	7.99	5.57
3 - Kansas River, KS	4.00	6.24	4.58	7.44	4.69
3 - Kansas River, KS	4.00	5.99	5.28	7.69	6.13
3 - Kansas River, KS	3.99	5.48	5.06	7.71	5.59
3 - Kansas River, KS	3.63	5.56	5.19	7.88	6.32
3 - Kansas River, KS	3.92	5.79	4.80	7.95	5.50
3 - Kansas River, KS	3.92	5.12	5.06	7.46	5.08
3 - Kansas River, KS	3.01	5.70	4.95	7.26	5.43
4 - Arkansas River, KS	5.98	8.88	6.91	9.17	7.72
4 - Arkansas River, KS	7.11	9.74	7.25	10.61	8.61
4 - Arkansas River, KS	5.88	8.88	7.73	11.01	7.92
4 - Arkansas River, KS	5.67	7.98	6.74	10.45	8.15
4 - Arkansas River, KS	6.07	8.86	7.33	10.98	6.46
4 - Arkansas River, KS	6.02	8.42	7.25	10.54	7.61
4 - Arkansas River, KS	6.20	9.80	8.44	11.56	7.99
4 - Arkansas River, KS	6.30	9.17	7.59	11.06	8.23
4 - Arkansas River, KS	5.58	8.34	7.37	10.43	7.94
4 - Arkansas River, KS	4.83	7.95	7.43	10.28	6.95
4 - Arkansas River, KS	6.05	9.19	7.97	10.28	8.93
4 - Arkansas River, KS	6.30	9.14	7.00	10.29	8.03
4 - Arkansas River, KS	6.05	7.06	6.96	9.60	7.17
5 - Pecos River, NM	6.12	8.10	6.44	9.74	8.30
5 - Pecos River, NM	7.16	9.14	7.30	10.29	9.06
5 - Pecos River, NM	6.09	8.93	7.15	10.30	8.45
5 - Pecos River, NM	6.37	9.32	7.49	10.73	8.75

Population	Head Width	Pectoral-fin Length	Pelvic-fin Length	Dorsal-fin Length (depressed)	Anal-fin Length (depressed)
5 - Pecos River, NM	5.79	8.13	6.48	9.75	8.07
5 - Pecos River, NM	7.25	9.88	7.74	11.35	9.17
5 - Pecos River, NM	6.24	8.58	7.44	10.24	8.38
5 - Pecos River, NM	5.41	8.24	6.36	9.26	7.94
5 - Pecos River, NM	6.06	8.17	6.13	9.31	8.06
5 - Pecos River, NM	6.54	9.34	7.46	11.40	9.63
6 - Canadian River, NM	5.62	7.89	6.71	9.97	6.81
6 - Canadian River, NM	5.15	7.76	6.27	9.07	6.83
6 - Canadian River, NM	5.37	7.54	6.18	8.76	6.99
6 - Canadian River, NM	5.51	7.58	6.24	9.01	6.80
6 - Canadian River, NM	4.85	6.27	5.54	8.28	6.27
6 - Canadian River, NM	5.47	7.33	5.90	9.12	6.49
6 - Canadian River, NM	4.86	7.58	6.03	9.13	6.90
6 - Canadian River, NM	4.79	7.34	5.50	8.96	6.56
6 - Canadian River, NM	4.64	5.88	5.04	8.05	5.65
6 - Canadian River, NM	5.53	7.63	6.62	10.01	7.02
7 - Sandy Creek, TX	5.97	7.42	6.80	10.19	8.18
7 - Sandy Creek, TX	6.52	7.42	7.85	11.61	8.83
7 - Sandy Creek, TX	6.62	6.79	6.85	9.72	7.73
7 - Sandy Creek, TX	6.32	7.52	7.45	9.53	7.53
7 - Sandy Creek, TX	6.14	7.95	6.88	10.46	7.50
7 - Sandy Creek, TX	5.28	6.56	6.35	8.56	6.53
7 - Sandy Creek, TX	6.32	8.27	6.93	10.61	7.42
7 - Sandy Creek, TX	5.68	8.12	7.31	9.87	7.63
7 - Sandy Creek, TX	5.57	6.86	6.41	10.01	7.45
7 - Sandy Creek, TX	5.62	7.05	7.21	9.23	7.75
7 - Sandy Creek, TX	4.57	6.51	6.10	6.66	4.11
8 - Frio River, TX	6.11	7.83	7.06	10.06	7.52
8 - Frio River, TX	4.32	6.23	5.83	6.48	5.79
8 - Frio River, TX	4.60	6.78	6.09	10.38	6.13
8 - Frio River, TX	4.59	6.57	6.00	10.00	6.22
8 - Frio River, TX	4.51	6.92	6.11	9.57	6.01
8 - Frio River, TX	5.79	7.29	6.45	9.85	6.68
8 - Frio River, TX	4.82	7.14	6.36	9.54	6.53
8 - Frio River, TX	4.76	7.53	6.61	10.27	6.23
8 - Frio River, TX	4.95	7.26	6.54	10.30	6.35
8 - Frio River, TX	5.84	7.11	6.71	10.76	6.66
9 - Clear Boogy Creek, OK	6.86	8.55	7.41	10.66	8.11
9 - Clear Boogy Creek, OK	6.57	8.40	7.95	10.21	7.66
9 - Clear Boogy Creek, OK	4.68	6.23	5.72	8.49	6.04
9 - Clear Boogy Creek, OK	4.07	6.25	5.00	8.00	5.45
9 - Clear Boogy Creek, OK	5.57	7.17	5.98	9.06	5.97
9 - Clear Boogy Creek, OK	5.21	6.90	5.36	8.06	5.53
9 - Clear Boogy Creek, OK	5.05	6.78	5.83	8.61	5.90
9 - Clear Boogy Creek, OK	4.82	6.28	5.86	8.50	6.41
9 - Clear Boogy Creek, OK	4.68	5.46	5.23	7.12	5.43
9 - Clear Boogy Creek, OK	4.17	5.31	4.61	6.90	5.26

Population	Snout Length	Orbit	Snout Depth	Transverse Head Depth
1 - Lake Creek, IA	3.53	4.64	2.38	6.15
1 - Lake Creek, IA	3.28	4.32	2.52	5.59
1 - Lake Creek, IA	3.53	4.88	2.37	6.10
1 - Lake Creek, IA	3.63	4.97	2.64	5.87
1 - Lake Creek, IA	3.54	4.69	2.27	6.06
1 - Lake Creek, IA	3.26	4.27	2.09	6.39
1 - Lake Creek, IA	3.13	4.19	1.73	5.05
1 - Lake Creek, IA	2.82	3.91	1.87	4.55
1 - Lake Creek, IA	3.17	4.50	2.19	5.17
1 - Lake Creek, IA	4.03	4.95	2.85	6.18
2 - Platte River, NE	2.26	2.90	2.94	4.90
2 - Platte River, NE	2.78	3.09	3.16	4.81
2 - Platte River, NE	2.70	3.23	2.92	5.04
2 - Platte River, NE	2.93	3.28	3.42	5.11
2 - Platte River, NE	3.12	3.20	3.61	5.25
2 - Platte River, NE	3.21	3.65	3.49	5.60
2 - Platte River, NE	2.82	3.10	3.09	4.78
2 - Platte River, NE	2.68	2.88	2.81	4.65
2 - Platte River, NE	3.79	3.62	3.33	5.03
3 - Kansas River, KS	1.98	3.08	1.33	4.22
3 - Kansas River, KS	2.20	3.47	1.44	3.77
3 - Kansas River, KS	2.22	2.91	1.28	3.77
3 - Kansas River, KS	2.16	3.68	1.49	4.19
3 - Kansas River, KS	2.43	3.15	1.65	3.77
3 - Kansas River, KS	2.20	3.10	1.42	3.65
3 - Kansas River, KS	1.64	3.42	1.26	3.73
3 - Kansas River, KS	2.10	3.12	1.32	3.80
3 - Kansas River, KS	2.00	2.66	1.25	3.02
4 - Arkansas River, KS	3.33	4.01	2.22	5.81
4 - Arkansas River, KS	3.02	4.26	2.54	6.90
4 - Arkansas River, KS	2.37	4.08	1.79	6.30
4 - Arkansas River, KS	2.85	4.14	2.38	5.58
4 - Arkansas River, KS	3.38	4.23	2.02	6.35
4 - Arkansas River, KS	3.15	4.62	1.86	5.67
4 - Arkansas River, KS	3.78	4.41	2.24	6.81
4 - Arkansas River, KS	3.27	4.70	2.31	5.89
4 - Arkansas River, KS	2.74	4.41	1.96	5.87
4 - Arkansas River, KS	2.65	4.18	1.80	4.90
4 - Arkansas River, KS	3.44	4.22	2.28	5.96
4 - Arkansas River, KS	3.47	4.25	2.07	6.72
4 - Arkansas River, KS	3.37	4.23	2.31	5.66
5 - Pecos River, NM	3.00	3.50	3.63	5.39
5 - Pecos River, NM	3.77	4.17	4.18	6.43
5 - Pecos River, NM	3.14	3.62	3.86	5.81
5 - Pecos River, NM	3.95	3.62	3.95	6.06

Population	Snout Length	Orbit	Snout Depth	Transverse Head Depth
5 - Pecos River, NM	3.02	3.43	3.46	5.24
5 - Pecos River, NM	4.02	4.16	4.30	7.02
5 - Pecos River, NM	3.47	3.62	3.81	5.82
5 - Pecos River, NM	2.95	3.34	3.44	5.21
5 - Pecos River, NM	3.09	3.50	3.59	5.58
5 - Pecos River, NM	3.49	3.56	3.78	5.82
6 - Canadian River, NM	2.71	3.30	3.18	5.07
6 - Canadian River, NM	2.74	3.44	3.06	4.62
6 - Canadian River, NM	2.82	3.24	3.16	5.43
6 - Canadian River, NM	3.10	3.29	3.18	5.11
6 - Canadian River, NM	2.51	2.92	2.64	4.47
6 - Canadian River, NM	2.88	3.20	3.04	5.36
6 - Canadian River, NM	2.69	3.16	2.68	4.69
6 - Canadian River, NM	2.83	3.18	2.82	4.90
6 - Canadian River, NM	2.09	2.87	2.48	4.19
6 - Canadian River, NM	2.97	3.33	3.13	5.28
7 - Sandy Creek, TX	3.54	4.20	2.46	6.22
7 - Sandy Creek, TX	3.66	4.51	3.05	6.61
7 - Sandy Creek, TX	3.67	4.39	2.79	6.19
7 - Sandy Creek, TX	3.23	4.46	2.28	5.99
7 - Sandy Creek, TX	3.40	4.26	2.50	6.07
7 - Sandy Creek, TX	2.50	3.83	2.06	5.00
7 - Sandy Creek, TX	3.16	4.32	2.45	6.45
7 - Sandy Creek, TX	3.33	4.21	1.90	5.73
7 - Sandy Creek, TX	3.32	3.85	2.26	5.22
7 - Sandy Creek, TX	3.33	4.18	2.14	5.36
7 - Sandy Creek, TX	2.52	3.32	1.61	4.47
8 - Frio River, TX	3.39	4.17	2.50	5.84
8 - Frio River, TX	2.12	2.96	1.93	3.82
8 - Frio River, TX	2.24	3.38	2.35	4.76
8 - Frio River, TX	2.31	3.34	2.14	4.36
8 - Frio River, TX	2.27	3.21	2.28	4.52
8 - Frio River, TX	2.33	3.91	2.32	5.02
8 - Frio River, TX	2.29	3.58	1.89	5.31
8 - Frio River, TX	2.24	3.46	2.07	5.40
8 - Frio River, TX	2.39	3.52	2.15	5.47
8 - Frio River, TX	2.71	3.68	2.26	5.69
9 - Clear Boogy Creek, OK	3.79	4.93	3.96	6.60
9 - Clear Boogy Creek, OK	4.01	4.62	4.19	6.51
9 - Clear Boogy Creek, OK	2.20	3.54	2.73	4.41
9 - Clear Boogy Creek, OK	1.98	3.00	2.56	4.01
9 - Clear Boogy Creek, OK	3.14	3.58	3.08	5.39
9 - Clear Boogy Creek, OK	2.29	3.29	2.62	4.56
9 - Clear Boogy Creek, OK	2.72	3.85	2.86	4.76
9 - Clear Boogy Creek, OK	2.79	3.36	2.56	4.89
9 - Clear Boogy Creek, OK	2.40	3.25	2.72	4.35
9 - Clear Boogy Creek, OK	2.49	3.13	2.55	4.21